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# Evaluation of Antipsychotic Activity of Some Indian Medicinal Plants

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**Abstract:** *Mental disorders such as schizophrenia and psychosis represent a significant global health burden, often requiring long-term pharmacological treatment. Although conventional antipsychotic drugs are effective, they are frequently associated with adverse effects such as sedation, weight gain, and extrapyramidal symptoms. This has led to growing interest in the exploration of safer and more effective alternatives derived from natural sources.*

*India possesses a rich heritage of traditional medicinal systems such as Ayurveda, which utilize numerous plant species for the treatment of neurological and psychiatric disorders. The present study focuses on the evaluation of antipsychotic activity of selected Indian medicinal plants, aiming to scientifically validate their traditional use. Various plant extracts were selected based on ethno pharmacological relevance and subjected to phytochemical screening to identify active constituents such as alkaloids, flavonoids, and terpenoids.*

*The antipsychotic potential of these plant extracts was assessed using established experimental models, including behavioral studies in laboratory animals, such as locomotor activity tests and conditioned avoidance response. The results indicated that certain plant extracts exhibited significant antipsychotic-like effects, possibly through modulation of dopaminergic and serotonergic pathways in the central nervous system.*

*These findings suggest that Indian medicinal plants may serve as promising sources for the development of novel antipsychotic agents with reduced side effects. However, further studies including isolation of active compounds, mechanism of action, and clinical trials are required to establish their therapeutic efficacy and safety..*

**Keywords:** *Mental disorders*

## I. INTRODUCTION

Every creature of God is an example of his existence, which cannot be built on its own. Human being is a complex creature, who has high intelligence and can perform multiple functions. It is made of multiple organ systems and each organ has its own function. But as a whole organism these organ systems work in accordance with others to maintain the homeostasis. Impairment in any part of the body leads to disturbance of the whole system and eventually loss of the homeostasis. This reduces the capacity of the human being and produces reduced or altered responses towards an external stimulus. A disease is any discomfort for the body part which deviates it from the regular functioning, which forces the body to change its normal functions in accordance with the changing needs. On a long run this deviation may be irreparable leading to loss or modification of normal body functions.

Among all the organ systems nervous system is regarded as the most complex one. It along with endocrine system maintains the body functions. Psychological and neurological disorders, which involve abnormality of nervous systems are the most disturbing disorders for all the age groups, as they alter the physical, mental and socio-economic status of the affected individual.

In the present stressful lifestyle, mood disorders like anxiety and depression are more common. They are known as psychological disorders as they influence the emotional and mental health of an individual. In a World Health report it is stated that people suffering from either of any mental or a behavioral disease is approximately 450 million, which is

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about 12.3% around the globe. Suicidal tendencies in psychiatric disorders is more which accounts for about 10 to 20 million every year.

The present situation in developing countries is such that, mental illness is given less importance due to the social stigma and also the fear of social isolation. Many of the active social organizations try to help them but without any success. Some of them come for the treatment but does not have enough knowledge and patience for the end results.

Anxiety is a state of normal response towards a stressful stimuli, a state which arises due to general or a specific stimulus which is thought to be life threatening in present or in the future situations. This creates a fearful state in the subject following the analysis made with respect to the stimuli, which increases the alertness and can progress with time to become severe. As the anxiety becomes more and more it becomes an anxiety disorder. It is to be noted that among the culture bound diseases which are enlisted in the appendix-I of DSM- IV, 40% of them have similar pathology as that of anxiety and this has been studied by research which provides a connection in these diseases and anxiety disorders.1.

Depression is a psychological disorder charecterised by number of feelings such as irritable mood, low self-esteem, sadness, helplessness, hopelessness, anhedonia, loneliness and restlessness etc. which dominate the life as it progress and makes difficult for the affected one to cope up with the life. It is the most common of the affective disorders and varies from milder to excessively depressed state charecterised by delusions, unrealistic imaginary thoughts etc. It is among one of the phychiatric disorder which can leading towards suicide of the affected person2.

Medical research provides new insights and better drugs for the treatment of disorders. Before they can be prescribed for the treatment they are tested in animals and human volunteers to find the beneficial effects. During this process some are proved to be better than the existing and some are left out vowing to their toxic effects or substandard effects than the existing drugs. Even with the advancements in the field of science natural way of treatment is considered as a better option even in the present situation.

All over the globe use of crude extracts from natural products for their therapeutic effects is followed from times immemorial. Most of the developing countries are still dependant on the age old medicinal practices for treating illness. Advancements in the field of medicine have led to the isolation of chemical constituents from these crude drugs which serve as lead compounds. Based on the lead synthetic derivatives are formed which increases the drug potency and action. Many of the obtained compounds from the different plant sources suach as yohimbine, physostigmine, opioids, muscarine, colchicine, cannabinoids, digitalis are important tools in physiological and pharmacological studies. Use of herbal medicines in most of the countries remain popular due to their less adverse effects and improved treatment for the chronic disorders. This has created demand for the high quality herbal medicinal products and also advancement in field of drug discovery has provided scientific evidences of treatment with these high quality herbal drugs.

Indian subcontinent is gifted with variety of medicinal plants which can be evidenced by the their use in traditional methods of practices like Ayurveda, Unani, Sidhha etc. These methods have provided usage of plant constituents in raw form and as extracts from them to treat various ailments. In the present study it is planned to evaluate the usage of folk medicines to treat disorders like anxiety and depression.

The selected plants Acorus calamus, Citrullus lanatus and Annona squamosa are used to cure various disorders in the folk medicine since from the old age. These plants contain chemical constituents like alkaloids, saponins, flavonoids, terpinoides, which are beneficial in treating psychotic disorders. As these plants are less explored for the anti-psychotic activities, they are considered for the present study.

Anti-anxiety and anti-depressant drugs which are available in the present market act by altering the monoamines, which are the root cause of the disease. Also these drugs have several limitations like adverse effects, therapeutic lag, low remission rates, physical dependence etc. So the experimental study aims to explore the beneficial effects of novel drugs to treat the disorders.

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## II. REVEIW OF LITERATURE

### 2.1 Acorus calamus:3

A.calamus (sweet flag or calamus) of family Acoraceae and genus Acorus is a monocot mostly dwelling in wetland and grows tall all over the year. It has 1 cm root which is thick and spreads under the soil. The roots are separated apart form the soil during collection and are size reduced to be stored.

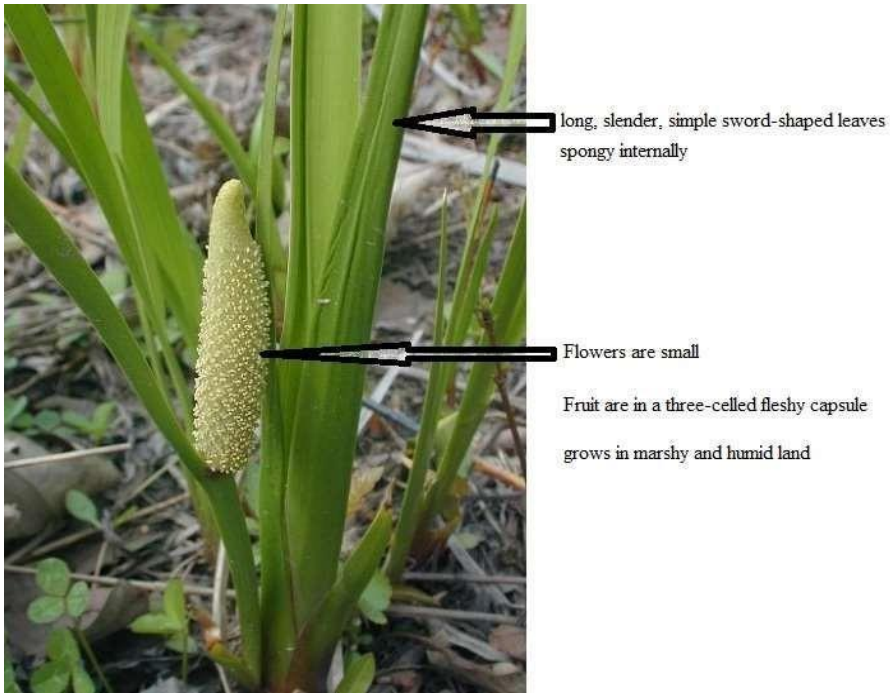


Fig 1:A calalmus plant

**Taxonomical classification**

| | |
|---|---|
| Kingdom | Plantae |
| Subkingdom | Tracheobionta |
| Super division | Spermatophyta |
| Division | Magnoliophyta |
| Class | Liliopsida |
| Subclass | Arecida |
| Order | Arales |
| Family | Acoraceae |
| Genus | Acorus L |
| Species | : Calamus |

**Vernacular Names**

| | |
|---|---|
| Arabic | Vaj, Vash, Oudul Vaj |
| Sanskrit | Bhadra, Bhutanashini, Vacha; |
| Hindi | Bach, Ghorbach, Safed bach |
| Gujarat | Gandhilovaj, Godavaj |
| Kashmir | Vachi, Vaigandar; |
| Urdu | Bach, Vaj |
| Kannada | Baje, Vasa; |
| English | Sweet flag, Calamus, Myrtle grass; |
| Unani | Vaj turki, Bacch |
| Ayurvedic | Vacha |

Table 1: Taxonomical classification and vernacular names of A calamus

Eastern Europe and Central Asia is the native place of A calamus and it is found throughout other countries like India, Indonesia, Sri Lanka, Northern Asia Minor, Japan, Europe, Southern Siberia, China, Burma.5

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Fig 2: A.calalmus rhizome

**2.1.1 Major chemical constituents:**

It has been reported that the plant possess mucilage, saponins, volatile oil, glycosides, polyphenolic compounds, tannins, flavonoids, and bitter principle acorine. The plant also contains glucoside, alkaloid, sesquiterpenes and essential oils i.e. pinene, calamen, clamenol, calameon, asarone, eugenol and camphene.4

The major phytochemical constituent presnt is β-Asarone. Along with them α- Asarone, elemicine, cis and trans-isoeugenol, cis-isoelemicine and their methyl ethers, camphene, acorone, acorenone, isohyobunones, acoragermacrone, 2-deca- 4,7-dienol, shyobunones, linalool, calamusenone and pre-isocalamendiol are also present.5 Phytochemical constituents such as triterpenes, flavonoids, mucilage, quinone, phenols, saponins, lectins, sugars, tannins and gums are reported to be present in various extracts of the rhizome. Roots are reported to contain sesquiterpenes like calamendiol, calamenone isocalamendiol. The phytochemical constituents present in the oil are phenylpropane and acoramone derivatives6.

Sweet flag has been used for its medicinal values in Chinese and Indian herbal traditions since from long time. In Ayurveda and Siddha system of medicinal practices, medicines prepared from the leaves, stem and roots are used to treat various diseases such as to counteract the side effects from the hallucinogens. It has carminative, diuretic and also laxative properties which is noted in the modern herbal medicine.

It is one of folklore herbs of Asian continent. The plant has been reported to have various CNS activities like reduction in spontaneous motor activity, amphetamine induced hyperactivity7are reported with alcoholic extract of this plant. The methanolic extract of rhizome has improved memory and cognition8, neuroprotective effect9, methanolic and ethanolic extracts reduced noise induced stress10 and hydro alcoholic extract have AchE inhibitory activity11.

It is grown wild or is cultivated. Cultivation of the plant is seen all over the Himalayas. The medicinal value of the aromatic oil in rhizomes attracts the plant cultivation for its commercial harvesting. Paithankar V V has reported that the rhizomes are used for the treatment of various gastro intestinal ailments like chronic diarrhoea, abdominal pain, dysentery, CNS diseases like epilepsy, mental disorders, also it has anthelmintic, carminative, nervine and stimulant properties and also acts as antispasmodic and expectorant12.

The extract of A calamus is also explored for other activities like anti diabetic, anti hypertensive, anti HIV, neuromodulatory, anticancer, antioxidant, immunosuppressive, wound healing, coronary vasodilatory, anti spasmodic, anti diarroheal, anthelmintic, hepatoprotective, bronchodilatory, anti fungal, analgesic and antipyretic effects4.

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**2.2 A squamosa:**

Annona is native to India and tropical America. The fruits appear pine-cone due to knobby and thick upper layer which vary from 6 to 10 cm in diameter. It is commonly known as 'Custard apple' 13.

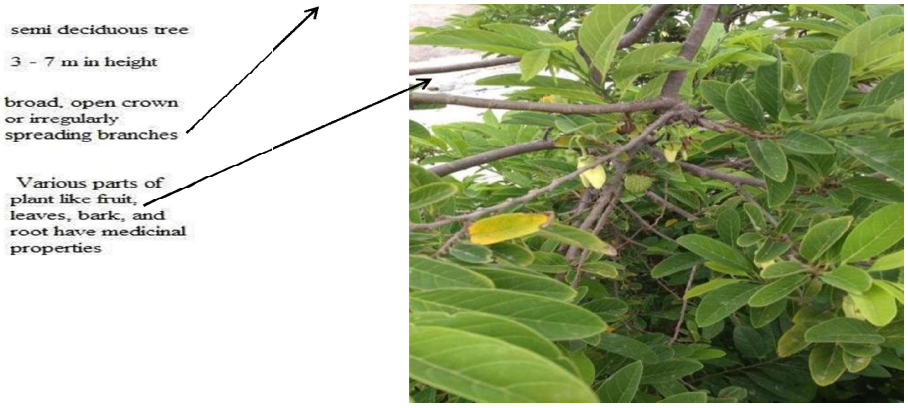


Fig 3: Annona squamosa tree with fruits

**Taxonomical classification**[14]

| | |
|---|---|
| **Kingdom** | Plantae |
| **Division** | Magnoliophyta |
| **Class** | Magnoliopsida |
| **Subclass** | Magnoliidae |
| **Order** | Magnoliales |
| **Family** | Annonaceae |
| **Subfamily** | Maloideae |
| **Tribe** | Abrae |
| **Genus** | Annona L |
| **Species** | *Annona squamosa L* |

**Synonyms**

| | |
|---|---|
| English | Custard apple |
| Hindi | Seetaphal |
| Sanskrit | Seetaphalam |
| Tamil | Sitapplam |
| Telgu | Sitaapandu |
| Kannada | Stephan |
| Bengali | Ata |

Table 2: Taxonomical classification and vernacular names of A squamosal

| S.No | Activity | Part used |
|---|---|---|
| 1 | Antibacterial and antiovulatory activities5 | Seed extracts Kannada |
| 2 | Anti hyper thyroidism6 | aqueous extract of the leaves |
| 3 | Anti-cancer activity | Ethanolic extract of leaves and stem |
| 4 | Free radical scavenging activity10 | leaf extract |
| 5 | Hypoglycemic effect11 | leaf extract |
| 6 | Hepatoprotective activity12 | leaf extract |
| 7 | Anti-parasitic activities (against lice)13 | seeds |

Table 3: Medicainal uses with the parts used of A squamosa .


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Traditionally the plant is used to treat fever, dysentery, cardiac problems, worm infestations, ulcers, dysuria, constipation, hemorrhage, epilepsy and has antifertility15 and antitumor16 activities.

The cyclic peptides present in the crude drug shows the following health benifits such as anti- diabetic, hypolipidemic, antitumor effect. It also has insecticidal, antioxidant and anti- inflammatory properties13.

The leaves infusion in a liter of water taken a cup after meal can protect from heart attack and also helps to relieve intestinal spasm by helping with proper digestion13.

The leaf decoction is proven to minimise dysentry and leaves applied over the wounds and ulcers after crushing them fastens the healing13.

In Northern India, Aligarh district villagers use young leaves with black pepper in diabetes which has proven to have good results in about 80% of the of population. The aqueous leaf extract has shown to provide beneficial effect in managing the increased cholecterol and triglyceride levels in diabetics and it has also provided with an enhanced insulin activity to lower the blood glucose levels13.

The bark decoction is given as a tonic and to halt diarrhea. Tropical Americans, use the leaf decoction as a remedy for cold, indigestion, as an febrifuge or to clear urine. Rheumatic pain and discomfort can be reduced by with taking bath with the leaf decoction13.

Sitopaladi churna is a popular remedy treating cold and cough13.

Phytochemical investigation of root and stem14 reveals the presence of Aporphine alkaloids17, flavonoids18, glycoside19, terpine derivatives and squamoline18.

It is reported that stem of A squamosa is isolated with six new ent-kaurane diterpenoids20.

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**Table 4: Chemical Constituents present in different parts of *A Squamosa* Linn[13]**

| S.No | Constituents isolated | Plant's Parts |
|---|---|---|
| | **Alkaloids** | |
| 1 | Anonaine | Leaves, tender stem, bark, roots, seeds. |
| 2 | Anolobine, Liriodenine, Norushinsunine, Reticuline | Roots. |
| 3 | Aporphine, Norcorydine, Norisocorydine, Norlaureline, Roemerine | Leaves, tender stem. |
| 4 | Corydine | Leaves, tender stem, bark. |
| 5 | Isocorydine | Leaves, tender stem, bark, roots. |
| 6 | Glaucine | Leaves, tender stem, bark. |
| 7 | Samoquasine A | Seeds. |
| 8 | Annosqualine | Stem. |
| **Cyclopeptides** | | |
| 9 | Cyclosqamosin A, B, C, D, E, F,G, H and I | Seeds. |
| 10 | Squamtin A, Annosquamosin A | Seeds. |
| **Acetogenines (Polyketide)** | | |
| 11 | Annonacin, Annonacin A, Annonastatin, Squamocin, Squamocin-O1, Squamocin-O2 | Seeds |
| 12 | Bullatacin, Bullatacinone | Stem bark |
| 13 | 4-Deoxyannoreticuin, Cis-4 deoxyannoreticuin, 2,4-Cis-squamoxinone, 2,4-Cis-Mosinone A | Bark |
| 14 | Mosin B and C | Bark |
| 15 | Squamotacin | Bark |
| 16 | Squamostatin C and D | Seeds |
| 17 | 2,4-Cis-bullatacinone | Seeds |
| 18 | Squamostatin C | Seeds |
| 19 | Annonin I, Annonin VI | Seeds |
| 20 | Squamostene-A, Reticulacin-1, Squamosinin-A, Annotemoyin-1, Notemoyin-2 | Seeds |
| **Ent-kauranedi-terpenoids** | | |
| 21 | Annomosin A | Stems |
| 22 | Annosquamosins A, B, C, D, E, F and G | Stems |

**2.1 Citrullus lanatus :**

C lanatus is a herb in the family Cucurbitaceae, contains 120 genera, the largest family distributed in the tropical countries with approximately 825 species. It contains important horticultural crops having sweet taste filled with good quantity of liquid.21 Watermelon is an annual herb, the seeds are flat and have oval texture, colour varies from yellow to lightish dark brown or lokking like black and are rarely white22.

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The Citrullus lanatus fruit has about 92% of water of the total weight with about 6% or the sugar and has vitamin A, B & C. The dried seed devoid of the shell contains 15.3 g of Carbohydrates, 47.4 g of fat, 28.3g of protein, 5.1g of water, 755 mg of phosphorous, 0.15 mg of riboflavin, 54 mg of calcium, 0.19 mg of thiamin, 3.55 mg of niacin, 58 µg of folate, 7.3 mg of iron and yields 2340 kJ energy22 per 100 g of fruit.

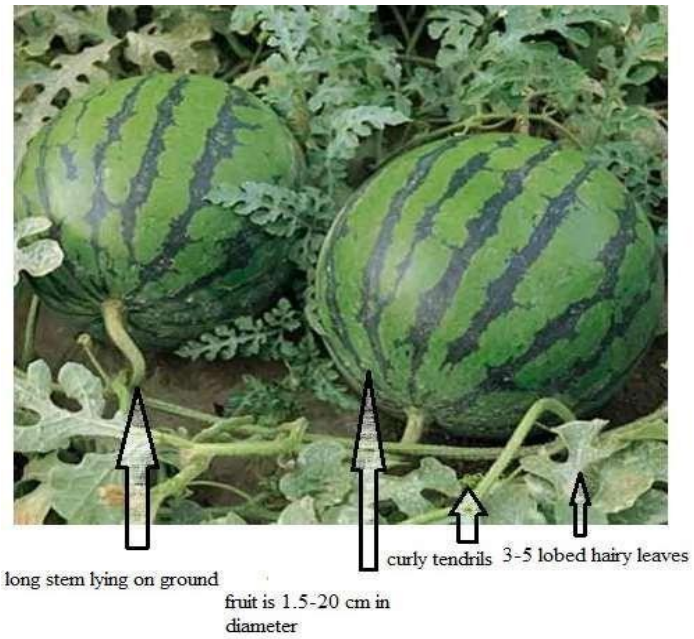


Fig 4: C lanatus fruits

**Botanical Description:** [23]

| | |
|---|---|
| Botanical name | Citrullus lanatus (Thunb) |
| Class | Equisetopsida |
| Kingdom | Plantae |
| Genus | Citrullus |
| Family | Cucurbitaceae |
| Order | Cucurbitales |

**Vernacular names:**

| | |
|---|---|
| Common name | Watermelon, Wild Watermelon |
| Local name | Tarbooz |
| English | Watermelon |
| Marathi | Tarbooz, Kalingad |
| Bengali | Tormuz |
| Malayalam | Thannimathan |
| Kanada | Kallagadi |
| Assamese | Tarmuj |
| Telugu | Pendalam |
| Tamil | Kizhangu |

Table 5: Botanical description and vernacular names of C lanatus.

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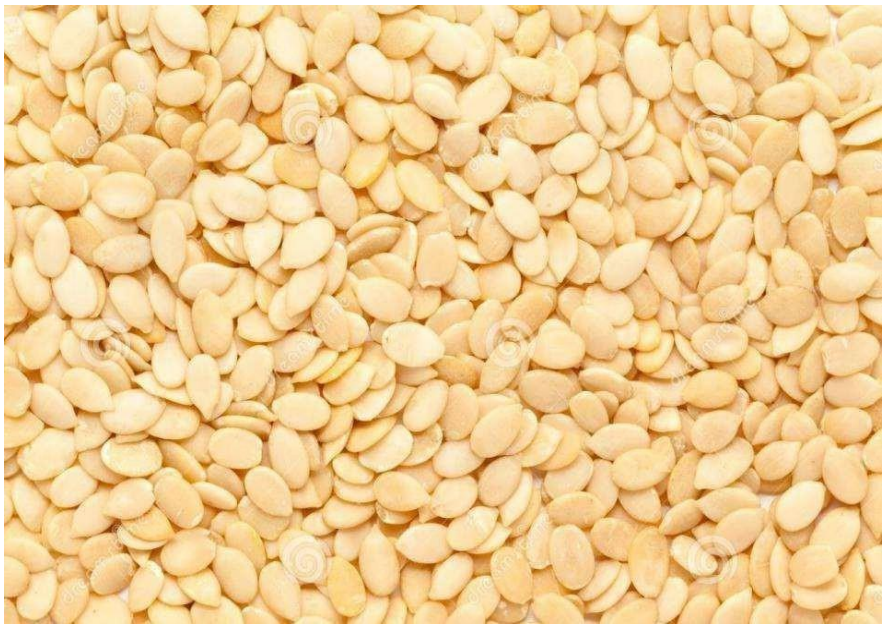

Fig 5: Citrullus lanatus seeds



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**Table 6:** Phytoconstituents of *C lanatus*[22]

| Part | Constituents |
|---|---|
| Seeds | alkaloids, flavanoids, tannins, amino acids, carbohydrates, cardioglycosides, terpenoids, steroids, carotenoids, oils and fats<br>Lycopene, ß-carotene, xanthophylls, phenolics, vitamin C<br>Protein-globulin, albumin, glutelin.<br>Flavonoids, vitamin C, thiamine, riboflavin, polyphenolic compounds<br>Glycoprotein-vicilin<br>2-dodecyclobutanon, 2-tetradcylcyclobutanon, cellulose radicals<br>Crude protein, carbohydrate, amino acids- arginine, isoleucine, leucine.<br>Mineral composition- Na, Ca, Mg |
| Seed oil | Total lipid content- oils- polyunsaturated fatty acids- oleic, linoliec fatty acid<br>High amount of higher fatty acids- palmitic acid, stearic acid, limoleic acid.<br>Unsaturated fatty acid- tocotrienols |
| Whole plant | Flavonoids, alkaloids, saponins, glycoside, tannins and phenols. |

The nutritional quality of watermelon shows that it is very rich in

- Vitamin which ranges between 1-3%
  - ✓ vitamin A 3%,
  - ✓ Thiamine (Vit. B1),
  - ✓ Riboflavin (Vit. B2),
  - ✓ Niacin (Vit. B3),
  - ✓ Pantothenic acid (B5),
  - ✓ vitamin B6 and
  - ✓ Folate (Vit. B9),
  - ✓ Vitamin C 14%.
- Minerals
  - ✓ Calcium 1%,
  - ✓ Iron 2%,
  - ✓ Magnesium 3%,
  - ✓ Phosphorus 2%,
  - ✓ Potassium 2% and
  - ✓ Zinc 1%.
- it also contains essential amino acids, oils and highly unsaturated fatty acids .

**2.2.1 Medicinal uses C lanatus:**

C lanatus has traditional value and is used to treat different disease conditions. It is a very important medicinal plant present in the Indian traditional medicine24.

Citrulline was the first amino acid to be extracted and analysed from the fruit.

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It has been reported by Chinmay that "the plant has hepatoprotective, antibacterial, antimicrobial, gastroprotective, antigiardial, antiulcer, analgesic, laxative, antifungal, antioxidant and antiinflammatory activities. Fruit is used in indigestion, as aphrodisiac and astringent to the bowels, acts as a diuretic, expectorant, stomachic and blood purifier. It decreases thirst and is good for sore eyes and as a brain tonic".21

**2.3 Anxiety:**

Anxiety state includes emotional, cognitive and beahvioural components which merge and results in a state of discomfort charecterised by increased panic. This affects the physical as well as the psychological status of the individual. There are numerous CNS disorders among which anxiety is the common disorder from which mankind is suffes a lot. In the few years of sucession the occurence of anxiety is increasing, as man is surrounded by a stringent lifestyle, which is rather competitive and inhumane and enforces stress at each step in life. Almost about 13% of the population are estimated to suffer from an anxiety disorder at any given time.

According to the WHO in 2015 around 3.6 % of total population were suffering with anxiety which is increased by 14.9% since from 2005 as a result of population growth. The suicidal deaths were around 7.8 lakhs and suicidal attempts were more than these during the year 2015.25 Anxiety is observed as an altered behaviour, emotion, physical and cognitive change in the affected person. Excessive dependance, need of help for any matter and avoiding to indulge in any situation can be seen as an altered behaviour. Emotionally the subject has excessive unstable thoughts and other physical changes like diarrhoea, hyperventilation, palpitations, tightness in chest, tremulousness can be observed. Cognitive change in anxiety is depicted by hypervigilance, worries, apprehension, thinking of harming and deep thinking about any subject.

Anxiety treatment options includes both non-pharmacological and pharmacological. However the main choice is pharmacological. Pharmacological treatment is a better option rather than psychosoical therapy. The present categories of anti anxiety and anti depressant drugs act by changing the neurotransmitters like GABA, serotonin and have provided good responses. Tricyclic antidepressants, serotonin reuptake inhibitors (SSRIs) and monoamine oxidase (MAO) inhibitors show benifit in anxiety too.

Benzodiazepines and its analogues are the most frequently prescribed anti-anxiety agents. Benzodiazepine usage can lead to include aggression, sedation, hyperactivity, impaired cognition, irritability and ataxia. These drugs when taken along with alcohol increases the effects of the later. Also the patient depend over them as they have a quicker action. As the therapy prolongs larger doses are required due to enzyme induction by them which can cause unwanted effects.

2.3.1 Classification of Anxiety disorders:

2.3.1.1 Generalized Anxiety Disorders (GAD)

2.3.1.2 Obsessive Compulsive Disorder (OCD)

2.3.1.3 Post-traumatic Stress Disorders (PSD)

2.3.1.4 Panic Disorders (PD)

2.3.1.5 Social Anxiety Disorder (SAD) and

2.3.1.6 Phobic disorders

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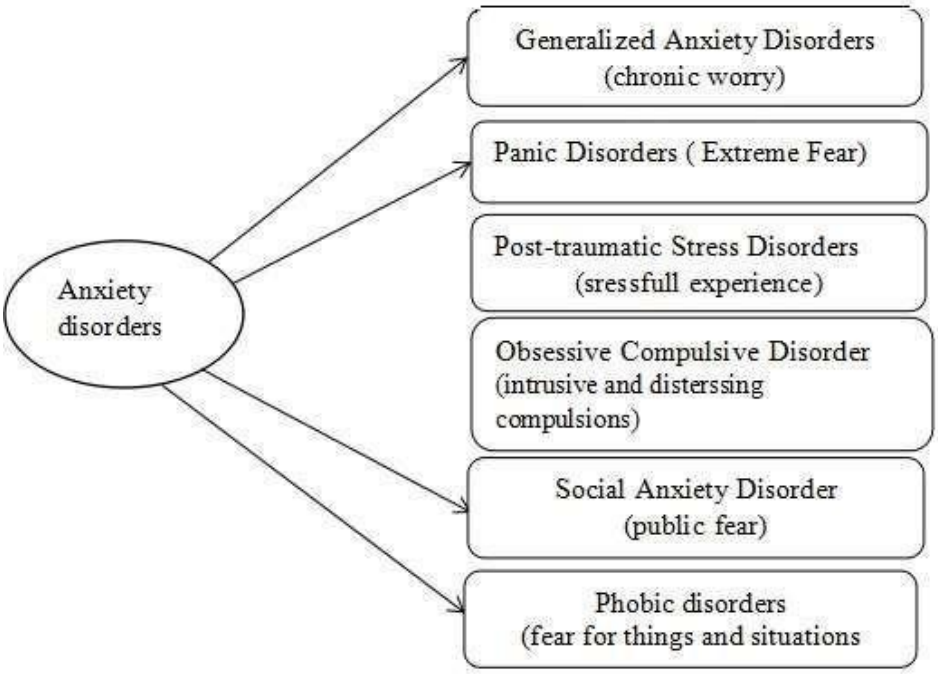


Fig 6. Six major types of anxiety disorders.[26]

**2.4.1.1 Generalized Anxiety Disorders (GAD): 27,28**

In this type of anxiety the person is anxious having with symptomps such as fear, nervousness, tremors, headache, gastric discomfort, muscle stiffness, palpitation, increased breathing, perspiration etc. These symptoms are moderate in nature. It is most common in females than males which may be due to excessive stress.

**2.4.1.2 Obsessive Compulsive Disorder (OCD):**

It is a state of person where in the subect goeson performing the repetitive mental and physical activities such as repetitive checks, walking up and down, asking the same thing again and again, washing of hands repetedly, talking with himself, repeated countings, begging for things repeatedly etc. All these obsessional acts are performed as a result of repetitive intutions the person has due to CNS activities. These obsessive acts can end up with other disorders like eczema, skin eruptions, hair loss due to hair pulling etc.26

**2.4.1.3 Post-traumatic Stress Disorders (PSD):**

It is a delayed response that occurs in succession with the stressful situation which is exceptional or catastrophic in nature such as sexual abuse, serious accident, natural disasters, criminal attacks, trauma etc., The symptoms include hyper arousal with hypervigilance, mood and cognition impairment, insomnia, emotional blunting, anhedonia. Concomitant use of alcohol and other medical conditions like dementia, diabetes mellitus accompany PSD.

**2.4.1.4 Panic Disorders (PD):**

In panic disorders the subject experiences severe chest pain, tend to choke, dizziness and has unrealistic thoughts which are recurrent and often unpredictable. Similarly the subject faces a fear of death tending him to loose control over normal things.

**2.4.1.5 Social Anxiety Disorder (SAD):**

Social Anxiety Disorder is an experience of being persistently judged negatively by the society in social or performance situations. Due to the fear affected individual avoids the social interaction, but when it is unavoidable he shows

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symptoms like trembling, blushing or nausea and it also recurs in stressful situations. DSM states that "the fear, anxiety and avoidance may persist for 6 or more months".

**2.4.1.6 Phobic disorders:**

Phobia is an increased response of fear towards a specific situation or an object. This provokes oneself to become isolated due to the anxiety associated with the phobic object. Usually phobias develop in the child hood and phobic anxiety often coexists with depression.

**2.1.1 Etiology of anxiety disorders:** The etiology and pathophysiology of these diseases can be explained by various cognitive, behavioral, biological, psychoanalytic and genetic theories29. These are Biopsychosocial factors which contribute to anxiety disorders.3

Table 7: Various causes of Anxiety disorder

| Biological causes | Psychological causes | Social causes |
|---|---|---|
| Heredity | Personality traits | Adverse Life Experiences |
| Neurotransmitter imbalance | Low self-esteem | Lack of social support |
| Illness | Cognitive dissonance | Work stress |
| Medications | Negative emotions | Lack of social skills |
| Nutritional factors | Inter and/or intra-personal conflicts | Changing values, Natural calamities |
| | Developmental crises | Conflict of societal norms |
| | Perception of situational factors | Terrorism |

**2.1.1.1 Biological factors:**

Genetic factors- The prevalence of anxiety in the family of individual suffering with anxiety is higher.

Imbalance in levels of neurotransmitters like nor-adrenaline, serotonin, GABA, dopamine etc. are linked to the neurobiology of anxiety.

**2.1.1.2 Psychological factors:**

Anxiety occurs if an individual does not cope up with the stress.

**2.1.1.3 Social factors:**

Loss of job, major illness, accidents, death of dependants, divorce may affect a person's perception of life and his response towards the everchanging life conditions.

A person is susceptible to one or the other anxiety conditions if he is repeatedly exposed to unnatural situations such as low economic status, poverty, violence, tensed life styles, terrorist attacks etc.

Diagnosis of anxiety disorders is a challenge to a physician as there are many ailments which have the same symptoms which are depicted in an anxety state by an individual. The following table-8 reflects it clearly.

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Table 8. Some medical diseases with anxiety like symptoms[31]

| | |
|---|---|
| Cardiovascular disorders | Angina, arrhythmias, congestive heart failure myocardial infarction, supraventricular tachycardia mitral valve prolapse |
| Endocrine and Metabolic disorders | Hyperthyroidism, hypoglycemia, Addison's disease Cushing's syndrome, pheochromocytoma, electrolyte abnormalities, hyperkalemia |
| CNS disorders | CNS tumors, dementia, migraine, pain, Parkinson's disease, seizures, stroke, multiple sclerosis, vertigo |
| Respiratory disorders | Asthma, pulmonary edema, embolus, pneumonia chronic obstructive lung disease |
| Gastrointestinal disorders | Crohn's disease, ulcerative colitis, irritable bowel syndrome |
| Others | HIV, systemic lupus erythmatosus, anemias |

There are different classes of drugs that cause anxiety like symptoms and have been tabulated in table-9.

Table 9. Different classes of drugs that cause anxiety like symptoms[31]

| | |
|---|---|
| CNS stimulants like | Amphetamines, caffeine, cocaine, ephedrine methylphenidate |
| CNS depressant like | Alcohol, anxiolytics, barbiturates, narcotic agonists sedative and hypnotics. |
| Cardiovascular drugs like | Captopril, enalapril, digoxin, reserpine, hydralazine |
| Others | Anticholinergics, anticonvulsants, antihistaminics NSAIDS, antidepressants, antipsychotics bronchodilators, steroids and thyroid preparations |

**2.1.1 Neuroanatomy of Anxiety:**

Noradrenaline (NA) cell bodies are higher in the locus cereleus (LC) part and its respose towards the sterss is remarkable. This is clearly observed in an experimentatiion witth cats where LC firing in cats does not increase if they face an usual confrontation such as a mouse, however if they are subjected for a threatful stimulus where mouse is replaced with a dog it increases the LC firing. Thus, threatful stimulus will activate the LC to release the neurotrnasmitters leading to as increased response towards the unnatural act of combat of danger. Further this LC activation can trigger hypothalamopituitary- adrenocortical (HPA) axis through the paraventricular nucleus (PVN) present in the hypothalamus which shows an increased stress response.

Also other brain areas are projected by noradrenergic neurons from LC and activate the hippocampus, the prefrontal cortex (PFC), thalamus, hypothalamus, amygdala, the periaqueductal gray (PAG) and the bed nucleus of the stria terminalis (BNST) which have a role in anxiety responses.

According to Gray's theory susceptibility to anxiety is due to individual differences in septohippocampal behavioral inhibition system (BIS) activity that includes the fight/flight system (F/FLS) and also the behavioral approach system (BAS) controlling the emotions. BIS compares actual with the expected stimulus and if there is any discrepancy BIS gets activated. It increases arousal and attention to the new environment and inhibits the ongoing behaviors. Thus, according to Gray "the anticipatory anxiety reflects a central state mediated by BIS activation, which is elicited by threats of punishment or failure and by novelty or uncertainty". The midbrain regions are activated through the amygdala which plays a prominent role in mediating fear and anxiety32 and it is the locus for anxiolytic drugs to mediate thier effects of modulating anxiety.33

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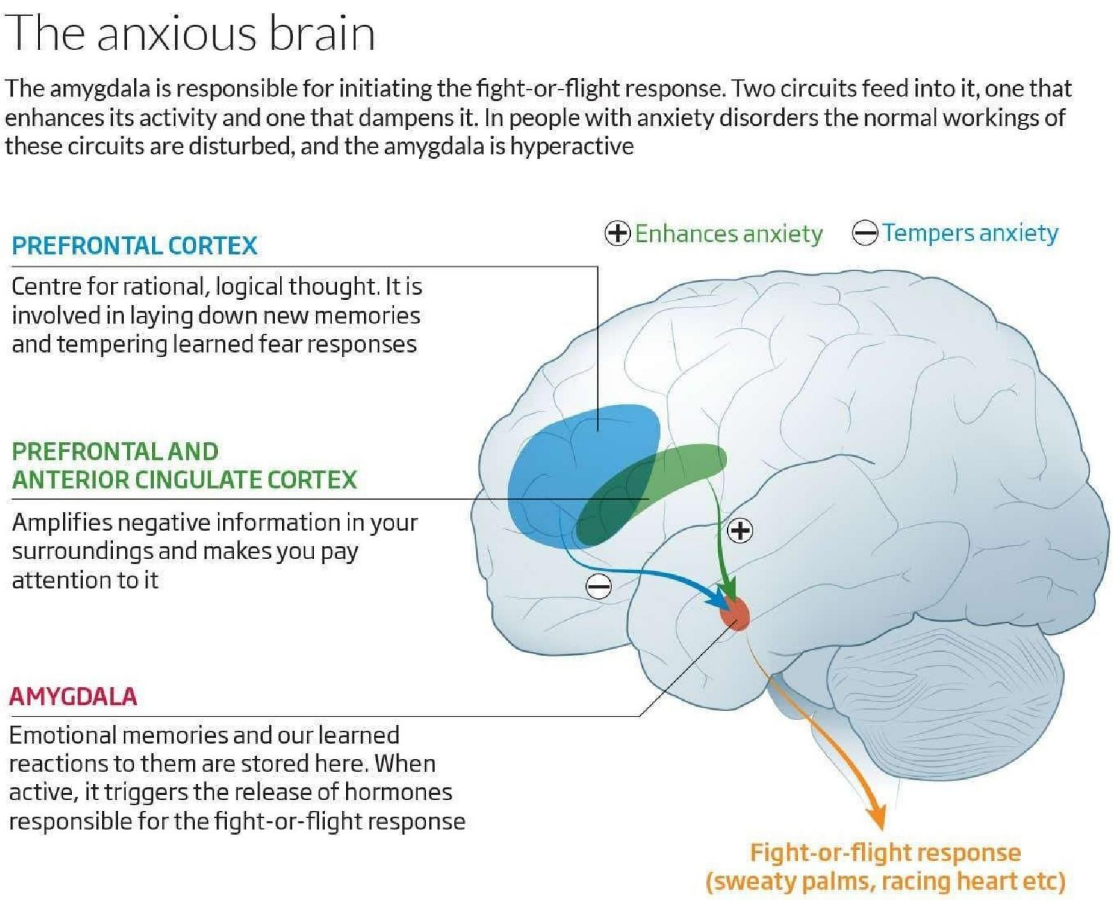


Fig 7: The anxious Brain

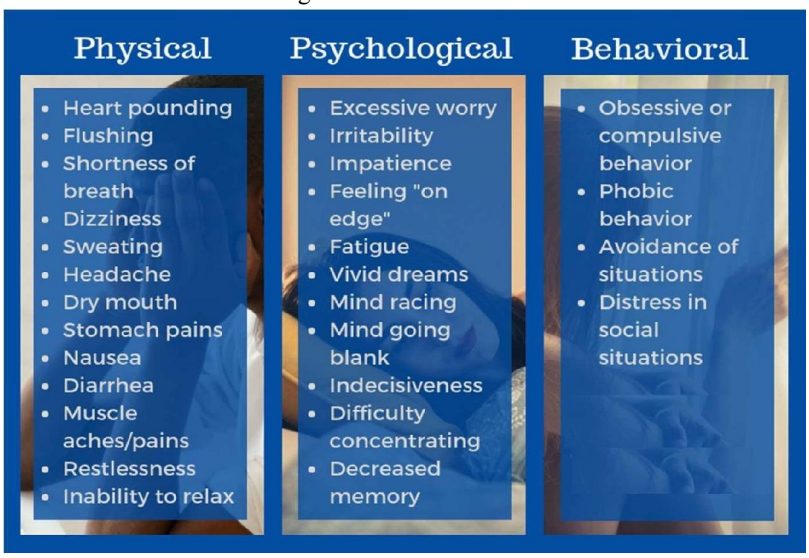


Fig 8: Symptoms of Anxiety

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**2.3.2 Biological modifiers of anxiety:26,34**

Anxiety, a mental disorder, caused as a result of imbalance in neurotransmitter levels in brain. Various neurotransmitters, neuropeptides, hormones affect the brain activity. Thus restoring the normal physiology of transmission by altering the abnormality is the main aim of drug treatment. The various modulators are as follows,

2.3.2.1 Nor adrenergic system
2.3.2.2 Acetylcholine
2.3.2.3 The GABAergic system
2.3.2.4 Cholecystokinin (CCK)
2.3.2.5 The serotonergic system
2.3.2.6 Atrial Natriuretic Peptide (ANP)
2.3.2.7 Hormones of Hypothalamic-Pituitary-Adrenal (HPA) axis 2.4.8 Cannabinoids

**2.4.4.1 Nor adrenergic system: 26**

In response to sterss and anxiety the NA levels are increased in various brain regions which play role in çontrol ling the anxiety behaviour. The neurotrnasmitter plays a vital role in maintaining the vigilant behaviour, attention, sleep etc. Yohimbine is anxiogenic in nature which acts by increasing the NA levels. It creates anxiety state after administration which indicates the role of NA in anxiety.

**2.4.4.2 Acetylcholine:34**

It has important role in learning and memory process. Anxioigenic and stressful stimuli enhances cholinergic input to hippocampus. Nicotine along with physostigmine reduces the anxiety which is mediated through the increase the acetylcholine level.

**2.4.4.3 The GABAergic system:34**

It çontrols the excitability states in all the brain areas and there is a balance between excitatory and inhibitory neurotrnamitters. If the GABA concentration is increased it leads to sedation, amnesia and ataxia. Similarly if the concentration reduces it leads to restlessness, insomnia, arousal, anxiety and exaggerated reaction.

**2.4.4.4 Cholecystokinin (CCK):36**

CCK has two types of receptors, one type found at peripheral brain parts is CCK–A and the other is present localised to brain is CCK-B. Among these CCK-B mediate anxiety responses which is evident by reversal of anxiety by its antagonists.

**2.4.4.5 The serotonergic system:26**

Serotonin is a natural mood stabilizer. It has various functions like regulation of mood, anxiety, happiness, sleep, bowel movements and sexual functions. It role in anxiety is by modulates the effect on locus cereleus and creates anxious state.

**2.4.4.6 Atrial Natriuretic Peptide (ANP):35**

ANP regulates the fluid and the electrolyte homeostasis. It inhibits the release of CRF and ACTH. Plasma ANP concentration and anxiety are inversely proportional. ANP acts as peripheral antagonist of CRF efffects but also in CNS it counteracts CRF's centrally mediated anxiogenic effects. Patients of alcohol withdrawal mostly have anxiety because it reduces the ANP levels. This is because there is a co-relation between alcohol seeking behaviour and plasma ANP levels.

**2.4.4.7 Hormones of Hypothalamic-Pituitary-Adrenal (HPA) axis:26**

Aging can withdraw the regulation of HPA axis by the PFC thus more cortisol is released resulting in aggarvation of anxiety. Along with this a lack or reduced fuctioning of compensatory protective effects which can reduce the levels of cortisol leads to appearance of cortisol symptoms which end up in anxiety. In old age anxiety normally the GAD is prevalent which is a result of increases levels of cortisol.

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**2.4.4.8 Cannabinoids:**

Cannabinoids are derived from Cannabis sativa which act through Cannabinoid type 1 (CB1) receptors. They decrease the neurotransmission of NA, glutamate and dopamine in the cortex and hippocampal regions of brain and thus interferes GABA transmission in frontal cortex, hippocampus and amygdala.37 Low doses of cannabinoids are anxiolytic but anxiogenic like behaviour is observed with higher doses. Inhibition of endocannabinoid degradation overcomes this dual response by enhancing CB1 receptor signalling, thus reducing the anxiogenic effects. Blockade or genetic deletion of CB1 receptor exerts anxiogenic likeeffects.38

Table 10: Neuromodulators, their action on respective receptors and their possible roles in anxiety modulation.[37]

| Neuromodulator | Anxiety modulation | Proposed mode of action |
|---|---|---|
| Acetylcholine | Anxiogenic<br>Anxiolytic | through M1 receptors<br>through facilitation of GABAergic influence |
| Adenosine | Anxiolysis | $A_1$ and $A_{2A}$ receptors (through GABA release) |
| Arginine Vasopressin | Anxiogenic | V (1B) receptors |
| Atrial Natriuretic Peptide | Anxiolytic activity | direct i.c.v. injection |
| Cannabinoid | Mixed profile | non-selective influence on glutamate norepinephrine and dopamine in hippocampus and cortex and interfere with GABAergic transmission in the amygdala, hippocampus |
| Cholecystokinin | Anxiogenic | CCK-2 receptors |
| Corticotropin releasing Factor (CRF) | Anxiogenic | CRH-1 receptors |
| GABA | Anxiolysis | Enhancement of GABAergic transmission |
| Galanin | Anxiolysis | Direct action in amygdala |
| Glucagon-like peptide –1 | Anxiogenic | Direct action in amygdala |
| Glutamate | Anxiogenic | Enhanced excitatory neurotmasmission |
| Melanin –Concentrating Hormone | Anxiogenic | MCH-1 receptor stimulation |
| Melatonin | Anxiolysis | GABA-A receptor stimulation |
| Neuroactive steroids | Anxiolysis | GABA-A receptor stimulation |
| Neuropeptide Y | Anxiolysis | Activation of Y1 and Y5 receptors |
| Noradrenaline | Anxiogenic | Stimulation of ß-receptors |
| Serotonin | Anxiogenesis | Activation of serotonergic neurons |
| Substance P and Tachykinin | Anxiolysis | NK1 receptor antagonism |

**2.1 DEPRESSION:**

Depression is mental state of prolonged illness observed with a sense of sadness and helplessness. It is seen with all the age groups and it abnormally affects the perception of an individual towards the life. Thus it affects the social and physical well being of an individual. At around 4 persons among 100 are suffering with one or other type of depression which may gradually increase in the next few years. It is one of the most prevalent mental disease and its occurrence is dependent on the events which happen just prior to the onset of depressive symptoms. 39

Depression is always associated with mortality and has a risk of over 20% in every ones lifetime. It can also be a risk factor in co-occurence of serious heath conditions such as diabetes, CVS diseases and will affect the prognosis of helthier life.40

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Table 11. Major classes of medications used for various anxiety disorders.[31]

| Class | Generic name | Used for | M.O.A. | Advantages | Limitations |
|---|---|---|---|---|---|
| Anticonvulsants | Gabapentin | SAD | Affect GABA | Usually effective within 2-4 Weeks | Sedation |
| Azaspirones | Buspirone | GAD | Enhances the activity of serotonin | Less sedating than benzodiaze-pines | Works slowly |
| Benzodiazepines | Lorazepam Clonazepam Oxazepam Diazepam Alprazolam | GAD, SAD, Panic Disorder | Enhance the function of GABA | Fast-acting, some people feel better the first day | Potentially habit-forming, can cause drowsiness, produce withdrawal symptoms, discontinuation should be done slowly |
| Beta blockers | Propranolol Atenolol | SAD | Blocks the action of adrenaline | Fast acting; non-habit forming | Should not be used with pre-existing medical conditions such as asthma, congestive heart failure, diabetes, vascular disease, hypothyroidism and angina pectoris |
| Monoamine oxidase inhibitors (MAO inhibitors) | Selegilene Isocarboxide Phenelzine Tranylcypromine | Panic disorder SAD PTSD | Block the effect of an important brain enzyme, preventing the breakdown of serotonin and Nor-adrenaline | Effective for many people, especially for patients not responding to other medications, 2-6 weeks until improvement occurs | Strict dietary restrictions and potential drug interactions, changes in blood pressure, moderate weight gain, reduced sexual response, insomnia |
| Selective serotonin reuptake inhibitors (SSRIs) | Citalopram Fluvoxamine Paroxetine Fluoxetine Sertraline | Panic disorder OCD SAD GAD | Affect the concentration of serotonin | Effective, with fewer side effects than other medications. 4-6 weeks until improvement occurs | Some people experience nausea, nervousness and diminished sex drive |
| Tricyclic antidepressants (TCAs) | Nortriptyline Amitriptyline Imipramine | Panic disorder PTSD OCD | Regulates serotonin and/or noradrenaline in the brain | Effective for many people, may take 2-6 weeks until improvement occurs | Dry mouth, constipation, blurry vision, difficulty urinating, dizziness, low blood pressure, moderate weight gain, sexual side effects |
| GAD = Generalized anxiety disorder, OCD = Obsessive compulsive disorder, PTSD =Post Traumatic stress disorder, SAD=Social anxiety disorder | | | | | |


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### 2.1.1 Symptoms of depression:

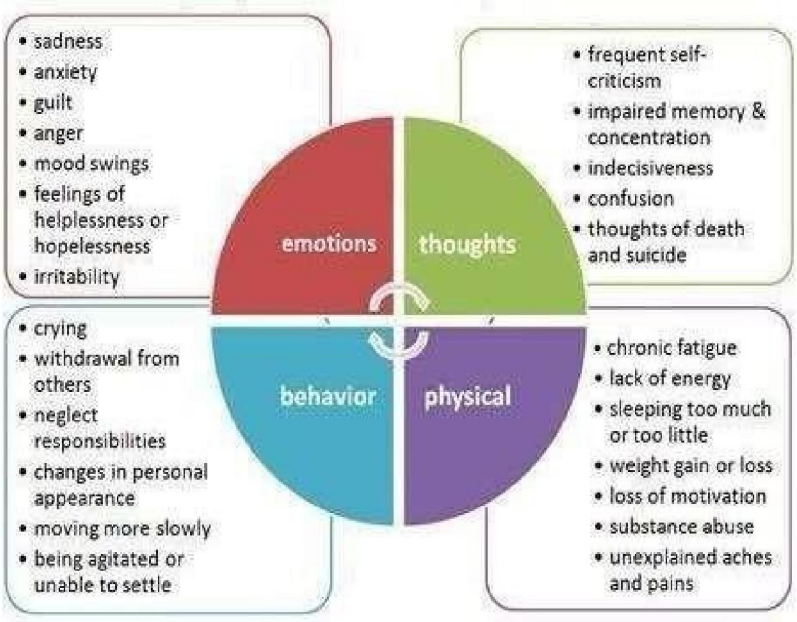


Fig. 9 Depressive symptoms

### 2.5.2 Classification of depressive disorders:

Table 12: Major types of depression[42]

| S No. | Type of Depression | Characteristics |
|---|---|---|
| 1 | Major depressive disorder | Recurrent expression of major depression, which is a common and serious illness. It can be mild, moderate or severe. Person with depression has low mood, anhedonia, negative cognition and disturbances in sleep, appetite and general activity for more than two weeks. |
| 2 | Dysthymia (minor depression) | It is chronic milder mood disturbance in which a person reports low mood daily for at least two years. These people are vulnerable to secondary episodes to major depression. |
| 3 | Bipolar depression | It was previously known as manic-depressive disorder. It is a condition in which depressive phases alternate with phases of mania or hypomania. Cyclothymia is a mild form of bipolar disorder charecterised by recurring episodes of hypomania and depression. |
| 4 | Melancholic depression | It is charecterised by a loss of pleasure in all the activities, failure of reaction to pleasure, depressive mood dominates than that of grief or loss. Early morning awakening, psychomotor retardation, excessive weight loss or guilt. |
| 5 | Atypical depression | It is associated with labile mood, hypersomnia, increased appetite, weight gain, heaviness in limbs, social impairment. |
| 6 | Catatonic depression | Disturbances of motor behaviour. Bizzare movements, immobility, stupor, purposeless movements are observed. |


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**2.3.3 Etiology:**

Depression is caused by an imbalance of neurotransmitters conveying and modulating mood signals in brain. Some of the common causes are

- serious medical conditions
- genetics (hereditary)
- alcohol and drug abuse
- trauma and high levels of stress
- postpartum depression (women may develop depression after the birth of the baby)
- mental illnesses like schizophrenia
- use of certain medications,
- individuals with low self-esteem

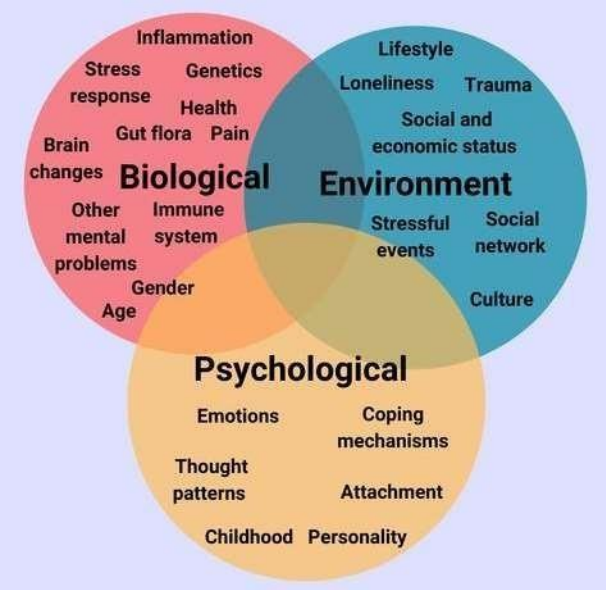


Fig 10: Causes of depression

**2.3.3.1 Genetic Causes of Depression:**

Research on genetic cause of depression have revealed functional polymorphisms (variation in DNA sequences) altering the normal expression and thus an abnormality in functioning is observed. Thus the progeny of the parents suffering with the genetic abnormality are at high risk to develop with the disease. In identical twins incidence of developing depression in both is 76% and if they are brought up in different environments then the incidence of developing is 67%. In fraternel twins it is 19%. This clearly indicates the role of genetic influence in acquiring the disorder.

**2.3.3.2 Environmental Causes of Depression:**

Environmental causes which are also referred to psychosocial factors include events such as stress, child abuse, trauma, loss of parents in child hood etc. Human reaction is a collection of experiences of thoughts, emotions and responses of past for a stimulus since from childhood. Stressful situations and development of depression are directly linked. Based on the situation for some people it may be positive and for some it may be negative. Child hood difficulties like sexual assault, physical abuse, parental separation, death of parents, mental illness of parents etc. can lead to the development of child hood depression. Natural disasters like earth quake, cyclones, floods, fire breakout could also add to the etiology of susceptible individuals towards depression.39


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**2.3.4 The neurobiology of depression:**

Genetic, epigenetic, biochemical and psychosocial factors are the biological basis of mood disorders. Recent advancements have explored the structure and relationship of various brain regions and the role of neuro modulators in mental disorders. This has helped to correlate various etiological factors. The abnormality in functioning of neuronal circuits in the brain may account for the disease progression. A change in brain structure and thus its functioning is observed in disease pathogenesis.43

**2.3.4.1 Prefrontal cortex: (PFC) 40**

The PFC lies anteriorly to the premotor and the primary motor area. The cingulate gyros connects the ventromedial (VMPFC) and the dorsolateral (DLPFC) with each other via the hippocampus. The VMPFC has role in expression of emotions, aggression, modulates pain, regulates neuroendocrine responses and the DLPFC analyses the emotional and behavioral outcomes towards a response and solves complex tasks and loss of this can lead to anhedonia which is prominent in depression.

**2.3.4.2 Amygdala.43**

The amygdala responds towards a surprising stimulus through neuroendocrine activation and also helps in learning. But an abnormal activation of this can lead to occurrence of depression.

**2.3.4.3 The hippocampus.43**

The hippocampus has following vital functions. Abnormality in functioning can lead unusual emotional responses. It provides inhibitory feedback to the HPA axis.

Table 13: Neurochemical/hormonal abnormalities in depression.

| Neurotransmitter | Levels |
|---|---|
| BDNF | Decreases |
| Proinflammatory cytokines | Increases |
| NA neurotransmission | Decreases |
| 5-HT neurotransmission | Decreases |
| Cortisol, CRH | Increases |

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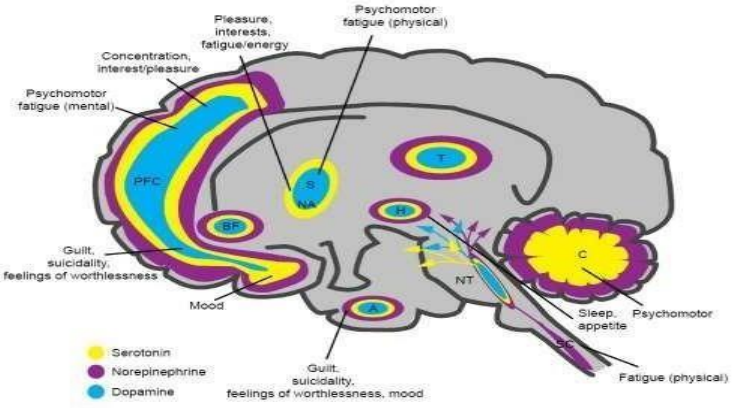
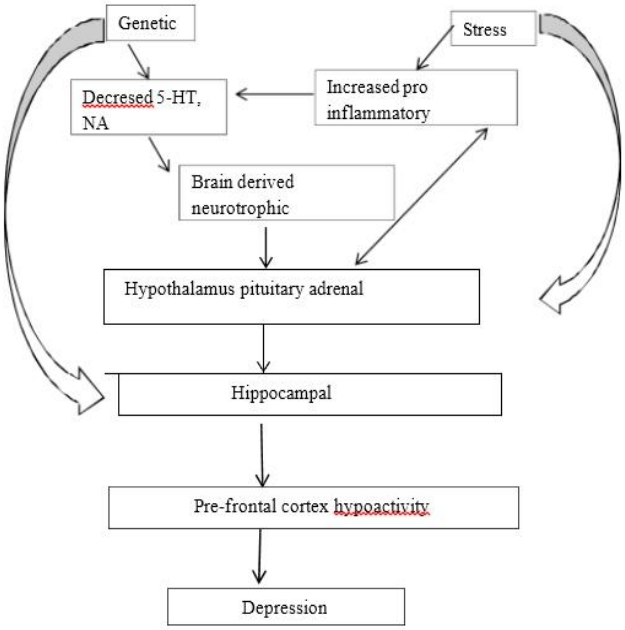



Fig 11 : Neurotranmitters and their hypothetically malfunctioning brain circuits associated with the diagnostic symptoms of depression.

A- Amygdala, BF- Basal forebrain, C- Cerebellum, H- Hypothalamus, NA- Nucleus accumbens, NT- Neurotransmitter centers, PFC- Prefrontal cortex, S- Striatum, SC- Spinal cord, T- Thalamus




Monoamine hypothesis:

A functional deficiency of neurotransmitters dopamine (DA), norepinephrine (NE) and 5- HT in the brain can lead to expression of depressive symptoms while an increase in these monoamines leads to mania. This is evidenced by the use of reserpine which can induce depressive like symptoms as it reduces the monoamine levels at the presynapse. Further the drug isoniazid can simulate maniac conditions as this increases the NE and 5-HT levels due to inhibition of mono amino oxidase.44

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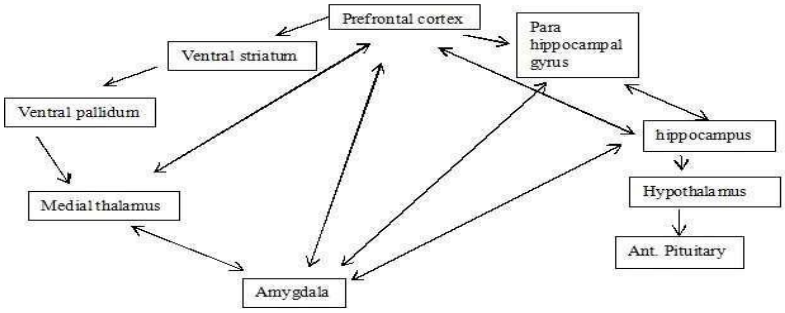


Connections between the pre-frontal cortex and limbic structures within the limbic-cortico-striato-pallido-thalamic circuits related to the medial and orbital prefrontal cortex implicated in depression.

A decrease in the inhibitory control of the limbic structures by the pre-frontal cortex is associated with cognitive, behavioural and other signs of depression as well as abnormalities in neuroendocrine function, pain modulation and neurotransmitter activity (affecting the raphe, serotonergic nuclei and noradrenergic nucleus ceruleus), through its connections with the hypothalamus and the midbrain, in particular the periaqueductal area.


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Table 14: Possible pharmacotherapeutic approaches for the treatment of selected symptoms in major depressive disorder (MDD) [43]

| Symptom | Pharmacotherapy |
|---|---|
| Anxiety* | • Buspirone • Citalopram • Escitalopram • Fluoxetine • Fluvoxamine • Lorazepam • Mirtazapine • Sertraline • Venlafaxine • Vilazodone • Vortioxetine • Doxepin |
| Cognitive problems | • Donepezil • Duloxetine • Galantamine • Methylphenidate • Modafinil • Vortioxetine |
| Insomnia | • Amitriptyline • Eszopiclone • Doxepin • Maprotiline • Mirtazapine • Nefazodone • Nortriptyline • Paroxetine • Ramelteon • Trazodone • Zaleplon • Zolpidem |
| Lack of energy /fatigue | • Atomoxetine • Bupropione • Desvenlafaxine • Fluoxetine • Modafinil • Sertraline • Venlafaxine |
| Pain | • Amitriptyline • Doxepin • Duloxetine • Milnacipran • Nortriptyline • Venlafaxine |
| Psychomotor problems | • Ziprasidone • Venlafaxine |
| Poor appetite/weight loss | • Mirtazapine |
| Sleepiness | • Atomoxetine • Bupropione • Modafinil |
| Note: *Most SSRIs are also approved by the US Food and Drug Administration to treat generalized anxiety disorder. Abbreviations: MDD, major depressive disorder; SSRI, selective serotonin reuptake inhibitor. | |

## III. RESEARCH METHODOLOGY

3.1 Objectives

The present study evaluates the possible anti anxiety and anti depressant effects using aqueous and ethanolic extracts of rhizome of Acorus calamus, roots of Annona squamosa and seeds of Citrullus lanatus in mice and rats with the following objectives:

3.1.1.1 To carry out collection and extraction (ethanolic and aqueous) of rhizome of Acorus calamus, roots of Annona squamosa and seeds of Citrullus lanatus.

3.1.1.2 To carryout qualitative phytochemical tests of obtained extracts for the detection of the various types of phytoconstituents.

3.1.1.3 To study the acute toxicity for determination of LD50 of all the extracts in mice and to select 3 different doses from each plant extract with respect to maximum LD50 dose tested subjected for experimental study.

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3.1.1.4 To evaluate the antianxiety activity of all the extracts on different animal models.
3.1.1.5 To assess the antidepressant activity of all the extracts in different animal models.
3.2 Statement of the Research Problem
The present research topic was focused to find out the rational usage of folkore medicines to treat the psychotic disorders like anxiety and depression.
3.3 Research Design
The proposed research work was an experimental study utilizing data obtained through the use of experimental animals.
3.4 Source of data:
The work has been planned to generate data from animal experiments in the laboratory i.e; using Albino Mice/Rats (either sex; weighing 20-24 g mice and 150- 250 g rats) as experimental animals in various models as described in various National and International Journals.
The scheme of the proposed work was as follows:
a) Collection of rhizome, roots and seeds respectively from the selected plants.
b) Dried under shade and powdered.
Preparation of different extracts with suitable solvents i.e., aqueous extracts of rhizomes of Acorus calamus (AQEAC), alcoholic extracts of rhizomes of Acorus calamus (ALEAC), aqueous extracts of roots of Annona squamosa (AQEAS), alcoholic extracts of roots of Annona squamosa (ALEAS), aqueous extracts of seeds of Citrullus lanatus (AQECL) and alcoholic extracts of seeds of Citrullus lanatus (ALECL).
c) Preliminary phytochemical testing of all the above extracts.
d) Study of the acute toxicity for determination of LD50 of all the extracts in mice upto the mximum dose of 2000 mg/kg.
e) Investigation of the antianxiety and antidepressant activities of all the extracts.

**3.5 Method of collection of data:**
The experiments were conducted using laboratory animals and the data was collected by analyzing the appropriate parameters. All experiments were conducted as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) norms after the approval of Institutional Animal Ethical Committee (IAEC).
Animals were housed in well maintained polypropylene cages with paddy husk as bedding material and a temperature of 25°C □ 2°C, 45- 55% of relative humidity for 12 h of light/dark cycle. Food with water was provided ad libitum in hygenic conditions.
**3.5.1 Plant material:**
Rhizomes of A calamus and seeds of C lanatus were procured from Bukkittu Santamma Wild life and ayurvedic commercial supplier in Raichur. A squamosa roots were procured from local fields in Raichur. The obtained plant materials were authentified based on the morphological characters by Dr. V Hemanth Kumar, Principal and Professor, Department of Pharmacognosy, V L College of Pharmacy, Raichur.
The whole study was divided into 3 phases.

**3.5.2 Phase I:**
**3.5.2.1 Preparation of various extracts.**
The collected plant material was shade dried and powdered. Powdered material was defatted with petroleum ether to remove fatty material and then subjected for soxhleation using alcohol and distilled water as solvents.
Coarsely powdered defatted plant material (250g) was packed in the extractor and the soxhelet apparatus was set. The solvent was added to the powder until it fills the siphon tube and then it was allowed for the extraction process in hot condition until the colour of the solvent in extractor was pale. The obtained extracts were concentrated on water bath in reduced temperature (< 50 ℃) and percentage yield was noted.
All the aqueous and alcoholic extracts were stored in tightly closed containers in cold conditions until use.

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### 3.5.2.2 Preliminary phytochemical screening 45,46.

Phytochemical investigation of different extracts was carried for detection of various phytochemical constituents by following standard procedures.

Table 15: Preliminary phytochemical screening of the extracts

| S.No | Test | AQEAC | ALEAC | AQECL | ALECL | AQEAS | ALEAS |
|---|---|---|---|---|---|---|---|
| **Tests for Alkaloids** The extract (50 mg) was stirred with few ml of HCl and filtered. The filtrate was tested with various alkaloidal reagents as follows: | | | | | | | |
| 1 | Mayer's test. Mayer's reagent + filtrate (creamy precipitate) | Alkaloids Present | Alkaloids present | Alkaloids present | Alkaloids present | Alkaloids present | Alkaloids present |
| 2 | Wagner's test. Wagner's reagent + filtrate (reddish-brown precipitate) | Alkaloids Present | Alkaloids present | Alkaloids present | Alkaloids present | Alkaloids present | Alkaloids present |
| **Tests for Carbohydrates** The extract (50 mg) dissolved in 3 ml of water and filtered. The filtrate was subjected to the following tests: | | | | | | | |
| 1 | Molisch's test 2 ml filtrate + α-napthol + 1ml $H_2SO_4$ Presence of violet ring | Carbohydrates Present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present |
| 2 | Barfoed's test 1 ml filtrate + Barfoed reagent (heated for 2 min) Presence of red precipitate | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present |
| 3 | Benedict's test 0.5 ml filtrate + Benedict reagent, heated for 2 min characteristic coloured precipitate | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present |
| 4 | Fehling's test 2 ml filtrate + Fehling reagent A & B, heated for 2 min. Presence of red precipitate | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present | Carbohydrates present |
| **Test for steroids and triterpenes** | | AQEAC | ALEAC | AQECL | ALECL | AQEAS | ALEAS |
| 1 | Extract + conc. $H_2SO_4$ Presence of rose red colored precipitate | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present |
| 2 | Salkowski's test Extract treated with $CHCl_3$ + conc. H2SO4 from the side wall of the test tube Presence of yellow coloured ring between junctions of two solutions observed which turned in to red coloured ring after 2 min | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present |
| 3 | Liebermann Burchard's test Extract treated with $CHCl_3$+ few drops of acetic anhydride followed by conc. $H_2SO_4$ from the side wall of the test tube Presence of violet to blue coloured ring between junctions of two solutions observed | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes absent | Steroids and Triterpenes absent | Steroids and Triterpenes present | Steroids and Triterpenes present |
| **Test for Saponins** | | | | | | | |
| 1 | Foam test: Extract shaken with water presence of foam observed | Saponins present | Saponins present | Saponins present | Saponins present | Saponins present | Saponins present |

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| Test for steroids and triterpenes | | AQEAC | ALEAC | AQECL | ALECL | AQEAS | ALEAS |
|---|---|---|---|---|---|---|---|
| 1 | Extract + conc. $H_2SO_4$ Presence of rose red colored precipitate | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present |
| 2 | Salkowski's test Extract treated with $CHCl_3$ + conc. H2SO4 from the side wall of the test tube Presence of yellow coloured ring between junctions of two solutions observed which turned in to red coloured ring after 2 min | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes present |
| 3 | Liebermann Burchard's test Extract treated with $CHCl_3$+ few drops of acetic anhydride followed by conc. $H_2SO_4$ from the side wall of the test tube Presence of violet to blue coloured ring between junctions of two solutions observed | Steroids and Triterpenes present | Steroids and Triterpenes present | Steroids and Triterpenes absent | Steroids and Triterpenes absent | Steroids and Triterpenes present | Steroids and Triterpenes present |
| **Test for Saponins** | | | | | | | |
| 1 | Foam test: Extract shaken with water presence of foam observed | Saponins present | Saponins present | Saponins present | Saponins present | Saponins present | Saponins present |

| Tests for Tannins | | AQEAC | ALEAC | AQECL | ALECL | AQEAS | ALEAS |
|---|---|---|---|---|---|---|---|
| 1 | Ferric chloride test. Extract + $FeCl_3$ formation of deep green colour | Tannins present | Tannins present | Tannins present | Tannins present | Tannins present | Tannins present |
| **Tests for Flavonoids** | | | | | | | |
| 1 | Shinoda test Extract + dil. HCl and Magnesium turning, presence of red colour | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present |
| | | | | | | | |
| 2 | Lead acetate + extract solution, white precipitate | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present |
| 3 | Zinc powder + dilcHCl + extract solution, presence of magneta colour | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present | Flavonoids present |
| **Tests for Proteins and Amino acids** The extract (50 mg) was dissolved in 5 ml of distilled water and filtered through filter paper. The filtrate was subjected for the following tests. | | | | | | | |
| 1 | Millon's test 2 ml of filtrate + few drops of Millon's reagent presence of white precipitate | Proteins and Amino acids absent | Proteins and Amino acids absent | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present |
| 2 | Biuret's test 2 ml of filtrate + 1 drop 2% Copper sulphate solution + 1 ml ethanol (95%) + KOH pellets Formation of pink colour | Proteins and Amino acids absent | Proteins and Amino acids absent | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present |


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| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 3 | Ninhydrin test<br>2 ml of filtrate + 2 drops of Ninhydrin solution. Formation of purple colour | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present | Proteins and Amino acids present |
| **Tests for glycosides** | | AQEAC | ALEAC | AQECL | ALECL | AQEAS | ALEAS |
| 1 | Baljet test<br>Solution A : 1 g Picric acid in 100 ml Ethyl alcohol solution B : 10 g NaOH in 100 ml distill water Both solutions combined and 2-3 drops of combined solution in 2-3 mg of test solution Formation of orange to deep red colour | glycosides absent | glycosides absent | glycosides present | glycosides present | glycosides present | glycosides present |
| 3 | Keller-Killiani test<br>extract 50 mg was dissolved in 2 ml Chloroform. $H_2SO_4$ was added to form a layer and the colour at interphase recorded. Presence of brown ring at interphase | glycosides absent | glycosides absent | glycosides present | glycosides present | glycosides present | glycosides present |

### 3.1.1 Phase II:

#### 3.1.1.1 Experimental animals:

For experimental study Albino mice (20-25 g) and Albino rats (Wistar strain) of either sex (150- 200g) were procured from Sri Venkateshwara Enterprises, Bengaluru. All the animals were allowed to adjust to the novel environment for 7 days period under standard husbandry condition as mentioned earlier. The animals were fed on with a standard pellet diet which was obtained from Amrut Laboratories and Pranav Agro Industries Ltd. Sangli (Maharashtra) and water allowed ad libitum under strict hygienic conditions. CPCSEA guidelines were followed for the experimentation (Registration Number 557/PO/Re/S/02/CPCSEA) and under the vigilance of Institutional Animal Ethical Committee (IAEC) of V.L. College of Pharmacy, Raichur, Karnataka.

#### 3.1.1.2 Determination of acute oral toxicity studies (LD50):

The acute oral toxicity of all the extracts were studied in female albino mice (20-25 g). Mice were grouped 7 groups i.e. normal çontrol , AQEAC, ALEAC, AQEAS, ALEAS, AQECL, ALECL and administered with 2000 mg/kg to extract on the respective groups. 3 h prior to the experimentation the animals were fasted and “Up and Down” procedure of OECD guidelines No.420 was adopted. Mortality, if any, was observed during the 48 h study period. The 48h test profile was based to choose the doses for further experimentation. Also a 14 days observance was followed to obtain long term toxicity of the drug. From the obtained LD50 results 1/20th (low) , 1/10th (medium) and 1/5th (high) doses were selected for the assesment.

#### 3.1.1.1 Grouping of animals:

For the experimental study animals were grouped as follows, Group I: çontrol ( treated with vehicle alone)
Group II: Standard (Diazepam 2 mg/kg)
Group III: Treatment with low dose (100 mg/kg) of AQEAC Group IV: Treatment with medium dose (200 mg/kg) of AQEAC Group V: Treatment with high dose (400 mg/kg) of AQEAC Group VI: Treatment with low dose (100 mg/kg) of AEAC Group VII: Treatment with medium dose (200 mg/kg) of AEAC Group
VIII: Treatment with high dose (400 mg/kg) of AEAC Group IX: Treatment with low dose (100 mg/kg) of AQECL Group X:
Treatment with medium dose (200 mg/kg) of AQECL Group XI:
Treatment with high dose (400 mg/kg) of AQECL Group XII:
Treatment with low dose (100 mg/kg) of ALECL
Group XIII: Treatment with medium dose (200 mg/kg) of ALECL Group XIV: Treatment with high dose (400 mg/kg) of ALECL Group XV: Treatment with low dose (100 mg/kg) of AQEAS Group XVI: Treatment with medium dose

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(200 mg/kg) of AQEAS Group XVII: Treatment with high dose (400 mg/kg) of AQEAS Group XVIII: Treated with low dose (100 mg/kg) of ALEAS
Group XIX: Treated with medium dose (200 mg/kg) of ALEAS Group XX: Treated with high dose (400 mg/kg) of ALEAS

**3.5.2.3 Preparation of dosage form:**
Drugs: Solutions of accurately weighed quantity of standard drugs Diazepam (2 mg/kg) and Imipramine (10 mg/kg) were prepared in distilled water by simple dissolution. Diazepam (Calmpose®) injection was diluted with distilled water to obtain a desired concentration. The extracts were suspended in distilled water with fine trituration and were administered immediately to the respective groups. The solution of standard and drug extract was freshly prepared on the same day prior to the dosing.
Oral route of administration was followed for dosing of standard and drug extracts. Volume of administration: The volume of drug to be administered was calculated based upon the body weight of animal.

**3.6 Determination of anti anxiety activity:**
To evaluate the anti anxiety activity the following animal models were used .
3.6.1 Staircase test in mice
3.6.2 Elevated plus maze test (EPM) in mice
3.6.3 Hole board test in rats
3.6.4 Light and dark model in mice
3.6.5 Open field behaviour in mice
3.6.6 Mirror chamber test in mice
3.6.1 Staircase test:47
The stair case is made of 5 identical steps and each step has a width of 10 cm, height of 2.5, and is 7.5 cm deep. The wall has similar height through out the apparatus. Albino mice of either sex with a body weight of 20-25 g were grouped as listed above. The respective groups received doses of vehicle, standard drug (Diazepam 2 mg/kg p.o.) and different doses of different extracts once daily for 7 consecutive days. On the 8th day 1 h after oral administration of vehicle/standard/extracts the mouse was subjected for the study by placing its back to the staircase. The following parameters were recorderd over a period of 3 min experimental session.
i. The total number of steps climbed
ii. The total number of rearings
Step is considered to be climbed only if the mouse has all its four paws over the step. Rearing is recorded if the mouse stands over its hind paws with front paws in air or against the wall. At the end of 3 min the stair case is to be cleaned with alcohol cotton so as to reomve any identifiable odours.
This test is rapid and does not produce any discomfort to the mice.
3.6.2 Elevated plus maze test:48
The apparatus is made of two open (16 cm x 5 cm) and two closed (16 cm×5 cm×12 cm) arm arranged in plus shape. A single central support elevates the set up to a height of 25cm above the floor. Albino mice of either sex with a body weight of 20-25 g were grouped as listed as earlier. The respective groups received doses of vehicle, standard drug (Diazepam 2 mg/kg p.o.) and different extracts at different dose levels once daily for 7 consecutive days. On the 8th day 1 h after oral administration of vehicle/standard/extracts the mouse was placed over the centre of the apparatus facing an open arm. The following parameters were observed during 5 min test,
i. Time spent in open arm
ii. Time spent in closed arm

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iii. Number of entries into open arm
iv. Number of entries into closed arm
v. Time spent in central platform
The entry of mouse in any arm is considerd only when it places all the four paws in the arm. After each trial the apparatus is wiped with wet cloth to remove odour and faeces.

**3.6.1 Hole board test:49**
The apparatus is a elevated four walled chamber (40X40X25 cm) having 16 holes of 3 cm diameter on floor. The elevation from the ground provides space so that the rats can poke through holes. Albino rats of either sex (150-250 g) were grouped as listed as earlier. The respective groups received doses of vehicle, standard drug (Diazepam 2 mg/kg p.o.) and different doses of extracts once daily for 7 consecutive days in respective groups. On the 8th day 1h after oral administration all the animals were subjected for the test. During testing the rat was placed on the floor of the apparatus and observed for the following parameters,
i. Time latency to the first head dip
ii. The number of head dips through the holes
iii. Total time spent with the head dips
iv. Number of rearings
An increase in exploratory behaviour is reflected by increased number and duration of head dips. Anti anxiety drugs tend to increase these parameters.

**3.6.2 Light and dark model:**
This model was proposed by Crawley and Goodwin and has been behaviorally, pharmacologically and physiologically validated. It is bases the fact that rodents tend to explore newer areas and avoid bright areas.50 These two parameters are most reliable to evaluate the anxiolytic effects of drugs which are being tested.51
It consists of a light (40×60×20 cm) and dark (40×40×20 cm) compartment. The light area was painted white and illuminated by 100 W table lamp and the other dark area was painted black. A partition separates these two compartments and a tunnel (7.5×7.5 cm) in the partition allows passage of mouse from one compartment to the other. Albino mice of both male and female sex with a body weight of 20-25 g were grouped as listed as earlier. The respective groups received doses of vehicle, standard drug (Diazepam 2 mg/kg p.o.) and different doses of extracts for 7 consecutive days once per day. On the 8th day 1 h after oral administration the mouse is placed in the centre of dark compartment facing towards the end wall which is opposite to the partition wall and the following parameters were recorded in a 5 min experiment session.52
i. The frequency of crossings in between the two compartments. (transitions)
ii. The total time of stay in the illuminated compartment
iii. The total time of stay in the dark compartment
iv. Time latency for the first crossing to the light compartment.
v. The frequency of rearings in the light part of the compartment.

**3.6.2 Open field behaviour:52**
The apparatus has a brightly illuminated arena in which the floor is divided into 16 squares of 15 cm2 a r e a . Among these 4 inner squares are present in the center and 12 squares are present at the periphery along the walls. Albino mice of both male and female sex weighing 20-25 g were grouped as listed as earlier. The respective groups received doses of vehicle, standard drug (Diazepam 2 mg/kg p.o.) and different doses of extracts daily once for 7 consecutive days. On the 8th day 1 h after oral administration test was started. Individual animals were placed in one of the corner squares at the periphery and following parameters were observed for 5 min as a test session.
vi. Number of rearings

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vii. Percentage of central locomotion
viii. Number of squares crossed
ix. Immobility time

**3.6.3 Mirror chambęr test:53**

It consists of a wooden box (40X40X30.5 cm) which houses an one side open cube like mirrored apparatus which is placed in the centre with a 5 cm of corridor at the surroundings.

To complete the cube a mirror is placed over the wooden wall facing the open face of the mirror chamber which induces a multiple object effect. Both male and female Albino mice of body weight of 20-25 g were grouped as listed above. The respective groups received doses of vehicle, standard drug (Diazepam 2 mg/kg p.o.) and different extracts daily single dose for 7 consecutive days. On the 8th day 1 h after oral administration individual animals were placed at fixed corners of the chamber and following parameters are noted for a period of 5 min test session.

i. Time latency of entry into the mįrrored chamber
ii. Number of entrįes into the mįrrored chamber
iii. Total time spent in the mįrrored chamber

**3.7 Determination of antidepressant activity:**

The following animal models were used to screen the antidepressant activity.

**3.7.1 Tail Suspęnsion Test (TST) 54:**

Albino micę of both the sęx with a body węight of 20-25 g were grouped as listed as earlier. The respective groups received doses of vehicle, standard drug (Imipramine 10 mg/kg p.o.) and different extracts oncę daily for 7 consecutive days. On the 8th day 1 h after oral administration mice were suspended 75 cm above the floor by the tail using a plastic string. The test is carried for a period of 8 min and duration of immobįlity is noted during the last 6 min period. Immobility of the animal is counted if it hangs without any motion.

**3.7.2 Forced Swim Test (FST): 54**

The apparatus is a box made of glass which is filled with water upto 15 cm. Albino mice of both the sex with a body weight of 20-25 g were grouped as listed as earlier. The respective groups receive doses of vehicle, standard drug (Imipramine 10 mg/kg p.o.) and different extracts daily one time for 7 consecutive days. On the 8th day 1 h after oral administration each mouse is subjected for test for a time period of 6 min by placing it in glass chamber.

The mouse is noted as immobile if it stands still in water without any motion and this immobilty time is noted in the last 4 min of the test.

**3.8 Statistical analysis of data:**

All the values were expręssed as mean ± SEM of the 6 animals in each group. The statistical differęnces in the mean values were analyzed using graph pad prism software. One way ANOVA (Analysis of Variancę) followed by Dunnętt's 't' test for comparison was used as a statistical test. 'p' value < 0.05*, 0.01** and 0.001*** were considered as statistically significant.

## IV. RESULT

In the SCT all the extracts have significantly increased the number of steps climbed and rearing frequencies. In the EPM the time spent in open arm is significantly increased after treatment with the standard drug and unit extracts also. In the HBT the number of headdips have been increased and in LDM the time spent in light compartment was significantly increased with the extracts. The number of crossings and rearing frequencies were increased in OFT also

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with a reduction in transfer latency and increase innumber of entries in the MCT after drug extract administration. The immobility time was significantly increased in FST and TST models.

## V. DISCUSSION

The Stair-case test is a simple and reliable method to screen of both anxiolytic and sedative properties of active chemical constituents and crude extracts in several laboratories. In a novel environment, rodents experience increase behavioral activity and vigilance. In the Stair case test, the step - climbing nature is considered to reflect the exploratory and locomotor activity and indicates the fitness of animal in a novel environment. The number of steps climbed and rearing were recorded for a 5 min period. The increase in the steps climbed denotes the motor coordination whcih reflects the alertness of brain and signifies that the drug is not a sedative.55 Also an increase in frequency of rearing is an indice of anti anxiety effect. The Diazepam (2 mg/kg ) and the extracts AQEAC, ALEAC, AQECL, ALECL AQEAS and ALEAS at various doses i.e. 100 mg/kg, 200 mg/kg and 400 mg/kg doses have shown a significant anxiolytic effect with increase in number of rearings and steps climbed. The EPM model utilizes the natural act of rodents to assess a newer environment and avoid open bright areas. In the EPM model animals are exposed to open area which would result to ambiguity either to approach or to avoid the situation.56 This is observed as an exploration or fear drive. The animals will experience fear due to the height and anxiety is observed (acrophobia). During the anxiety there is reduced motor activity which is reflected by number of transitions and time spent by the animal. The animal is allowed for exploration of the apparatus by placing it at the centre of the maze and where it can move along the four arms. Mice and rats normally prefer the dark area since they are nocturnals and the anxious rodents tend to spend less time over the open or bright lit areas. The treatment with standard drug AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS all these extracts with three different doses have significantly increased the number of entries and time spent into open arms.

The EPM model has varied advantages like it is quick, effective, requires no prior training and provides with no discomfort to the animal. Diazepam is used as a standard drug as the parameters like number of entries and time spent in open arms are sensitive to drugs acting at GABAA receptor. Anxiolytic compounds decrease number of entries and time spent in closed arm and increase the open arm exploration time. The treatment with standard drug AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS all these extracts with three different doses have significantly increased number of entries and the time spent into open arms.

Hole-Board experiment introduced in 1962 is used to evaluate anxious and exploratory behavior of laboratory rodents, used to confirm the anxiolytic and sedative action of any drug or extract.57 In this test, the latency and frequency of head dip by the experimental animal is observed. Anxiolytic drugs tend to reduce the latency and increase the frequency of headdippings. A decrease in the frequency of head dipping denotes the sedative activity of the drug under test. The head dipping behaviour is a measure of neophilia. In this model the standard drug (Diazepam 2 mg/kg) and AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS treatment with three different doses have shown a significant reduction in head dips duration, the time latency of first head dips and number of head dips and increased the number of rearings.

Craw-ley and Goodwin described Light and dark exploration test in 1980. It is a simple behavioral which detects the anxiolytic action of test compounds50. The anxious state of the animal is depicted by its choice for the dark compartment58. This model is based on the fact that rodents prefer dark area and also avoid illuminant areas, tend to explore novel surroundings.

An increase in time latency for entry into the dark compartment, a decrease in frequencies of entry in dark compartment and increase in time spent in light compartment exhibits anxiolytic property of the test drug. In an anxiety state rodents have less rearings but anxiolytic drugs tend to increase frequency of rearings59.In the light and dark model it was found that the time spent in light compartment, number of crossings have been significantly increased with standard drug treatment and treatment with all the three doses of AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS. The

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rearing frequency was significantly increased with AQEAC and ALEAC, medium and high doses of AQECL and high doses of ALECL, AQEAS and ALEAS.

In 1934 Calvin Hall invented open field test used to evaluate sedative and anxiolytic properties of the test drugs. The technique describes the animal behavior in a state of anxiety. Rodents placed in a new environment may experience signs of anxiety such as decreased mobility, exploration, grooming and rearing with concurrent increased urination and defecation. An increase in mobility and rearing and alertness of the animal exhibits anxiolytic activity of the test drug 60.An increase in line crossings and number of rearings indicate an increase in locomotor activity and exploration and low anxiety61.

In the Open field after treatment of standard drug and all the three doses of AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS test there was a significant increase in total number of crossings, peripheral square crossings. The rearing frequencies have been significantly increased only with low, medium and high doses of AQEAC, ALEAC, medium and high doses of AQECL and low, medium and high doses of ALECL, AQEAS, but ALEAS has not shown non any significant effect.

The mirror chamber is developed to simulate the novel environment. The animal generates anxiety due to ambiguous state i.e. either to approach or to avoid the novel area The reflected images in the mirror might also serve as a source of anxiety. Mirror Chamber model is based on the fact of introducing the rodent in a mirrored area exhibits an avoidance response. The anxiety is observed by the avoidance response towards the stimulus. Further in the Mirror chamber the administration of standard and AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS at three different dose levels have exhibited a significant reduction in latency to enter into the mirror chamber and an increase in the number of entries and time spent in the mirrored chamber.

Anxiolytic action correlates with increased GABA content in cerebral hemisphere as these receptors are related to anxiety and direct activation of these leads to anxiolytic effect. Many of the CNS chemical mediators are responsible for anxiolytic action hence it is very difficult to explain a single mechanism for anxiolytic effect.

It is also reported that the interaction of drugs with neural substrates or chemical mediators like NA, serotonin, GABA, hormone testosterone and Magnesium or natural endogenous mediators of the body which are responsible for aggressive and anxiety conditions.

Depression is charecterised by low mood and reduced interest towards normal activity which negatively effects a person's feelings, thoughts, physical well-being, observance, behaviour etc.62 Major depressive disorder is related to changes in brain neurotransmitter, like 5-HT, norepinephrine (NE) and dopamine. At about 9.5% of women and 5.8% of men experience the depressive episodes during a lifetime with an increased suicidal tendencies.

The present study is designed to screen the antidepressant activity of rhizome extract of Acalamus, seed extracts of C lanatus and root extracts of A squamosa . Experiments were designed by different tests such as Forced swim test (FST) and Tail suspension test (TST). In both the models a normal animal is subjected to an unavoidable aversive situation like immobility and agitation. The animal stays immobile so as to conserve enegy and agitation is to search for escape and survival, it is highly energy consuming. Even in desperate situations the antidepressant treatment makes the animals to uphold the struggle in unavoidable situation and thus the animals show less immobility.

In the FST and TST test models there is a significant increase in immobility time with administration of standard drug and all the three doses of AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS.

## VI. SUMMARY

The present work was carried to evaluate the anti-anxiety and anti depressant properties of aqueous and alcoholic extracts of rhizome of Acalamus , seeds of C lanatus and roots of A squamosa. The crude drugs were obtained from local ayurvedic trader and were authenticated, dried and after size reduction were subjected for extraction with ethanol and water by soxhlation. The obtained filterate was concentrated and subjected for acute oral toxicity studies following OECD guideline 420. From the obtained results with respect to highest LD50 dose testeed i.e. 2000 mg/kg doses were selected and fixed as low 100 mg/kg , medium 200 mg/kg and high 400 mg/kg doses respectively.

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The preliminary phytochemical screening revealed the presence of alkaloids, steroids, saponins, flavonoids, Carbohydrates and terpenoids. The extracts were subjected for evaluation of anti-anxiety and antidepressant activities using the following models.

1. Staircase test in mice
2. Elevated plus maze test (EPM) in mice
3. Hole board test in rats
4. Light and dark mode in mice
5. Open field behaviour test in mice
6. Mirror chamber test in mice
7. Forced swim test in mice
8. Tail suspension test in mice

In the Stair Case test AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS with all the three doses have shown a significant increase in number of steps climbed and number of rearings.

In EPM, AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS all the extracts with different doses have significantly increased the time spent and number of entries into open arms.

In Hole Board model AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS all the extracts with three different doses have shown a significant reduction in the time latency of first head dips and increased the number of rearings, head dips and duration of heąd dips.

In the light and dark model it was found that the time spent in light compartment, number of crossings have been significantly increased with standard drug and also with three doses of AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS. The rearing frequency was significantly increased with AQEAC and ALEAC, medium and high doses with only AQECL and high doses of ALECL, AQEAS and ALEAS.

In the open field apparatus test was a significant increase in total number of crossings, peripheral square crossings with treatment of standard drug and all the three doses of AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS. But the rearing frequencies have been significantly increased only with low, medium and high doses of AQEAC, ALEAC and ALECL, medium and high doses of AQECL, AQEAS and ALEAS has not shown any significant effect.

Further in the Mirror chamber test AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS with three different doses exhibited a significant reduction in latency to enter into the mirror chamber and an increase in the number of entries and time spent in the mirrored chamber.

In the FST and TST test models there is a significant increase in immobility time with administration of standard drug and all the three doses of AQEAC, ALEAC, AQECL, ALECL, AQEAS and ALEAS.

## VII. CONCLUSION

• The evaluation of anti-anxiety and anti depressant properties of aqueous and alcoholic extracts of rhizome of A calamus , seeds of C lanatus and roots of Asquamosa in mice and rats with various experimental models has revelaed that when compared with the control groups the selected crude drug extracts posess a significant activity.

• The acute oral toxicity studies have shown no mortality with any of these extracts.

• Aqueous and alcoholic extracts of all the plants exhibited anxiolytic and anti depressant activites.

Table 40 :Phytoconstituents obtained from various plant sources reported for their anti-anxiety and antidepressant activities

S. No. Botanical name Chemical constituent Reported activity

1 Actaea racemosa L Triterpenoids and derivatives of flavonoids Anti- anxiety and antidepressant activities61

2 Cassia siamea (Caesalpinaceae) Phenols (Barakol) Anti-anxiety61

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3 Passiflora incarnate
(Passifloraceae) flavonoids Anti-depressant62
4 Bacopa monniera
(Scorphulariaceae) Saponins Anti-depressant62
5 Withania somnifera
(Solanaceae) Alkaloid Anti-depressant63
6 Centella asiatica Triterpenoids and saponins Anti-anxiety64
7 Curcuma longa Phenols Anti-anxiety64

• It has been well investigated that crude plant extracts which posess the chemical constituents as shown in table 40 provide a beneficial effect in reducing the severity of the disease. Thus the obtained results may be attributed to the presence of the phytoconstituents in the crude drug extracts.

• Among the three crude drugs the AQEAC and ALEAC has better activity than the other two drugs. This might be due to the quantitative variation in constituents among the extracts which need to be further investigated.

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LIST OF PAPERS PUBLISHED/ACCEPTED/COMMUNICATED

1. Md Shamsuddin Munawar and Dr. N Venkat Rao. Evalution of anti-anxiety activity of seed extrats of citrullus lanatus linn in rats and mice. J Emer Tech Inno Res.2019:6(1).
2. Md Shamsuddin Munawar and Dr. N Venkat Rao. Screening of anti-anxiety activity of seed extrats of citrullus lanatus linn in rats and mice. Inter J Res Analy Rev. 2019:6(2).

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LIST PRESENTATIONS/CONFERENCES ATTENDED

1. Presented a poster entitled “Anti anxiety activity of Citrullus lanatus Linn in mice” at 15 th Indo African conference held on 19 September 2018 at Vaagdevi Pharmacy College, Warangal, Telangana.
2. Participated as a delegate in KAAPTICON-2018 held at N E T pharmacy college, Raichur on 26-27 October 2018.
3. Presented a oral paper entitled “Anti anxiety activity of Acorus calamus in mice and rats ” at Dravyaka 2019 held on 13-14 November 2019 at Geetanjali college of Pharmacy, Cheeryal, Telangana.