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Antidepressant Activity of Ethanolic Unripe Fruit Rind Extract of Limonia Acidissima Linnby Using Albino Mice"

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ABSTRACT:
Limonia acidissimia linn commonly known as wood apple, which is traditional plant and has many medicinal and cosmetic properties. Which cures cardiac problems, diarrhoea, dysentery, cough, anti-inflammatory, anti-mitotic, anti-microbial property. Chemical constituent which are found to be flavonoids, saponin, glycosides, tannins, coumarin. It belongs to the genus aeglesmarmelus and family Rutaceae. In India they may be found in Punjab, Rajasthan, Madhya Pradesh, West Bengal, Goa, and Karnataka. Unripe fruits are sour aromatic, astringent, constipating, removes itching etc. It may be used to treat skin cancer. Depression is one the most common disorder which is caused due to imbalances between neurotransmitters such as NE, Dopamine and Serotonin, which is characterized by lack of interest, energy, concentration, loss of appetite, poor sleep and suicidal tendencies. Which disrupt social life, career, as well as personal life. The antidepressant activity is studying by using behavioural tests such as FST, TST in albino mice. The total a30 no. of animals about (15-19) gm. are required to perform the test. The animals are divided into 4 groups. Group-1 standard (imipramine), group-2 control (saline), group-3, 4, are used as test animals with different concentrations of test drug. In both the test immobility of animals should observe. This research shows the unripe fruit rind extract has the antidepressant activity which is observed in albino mice.

KEYWORDS: Rutacea, FST, TST, Neurotransmitters.

I. INTRODUCTION

Depression is an umbrella term used to describe a wide range of disorders that affect mood, body function and behaviour. It is not only caused by lifestyle changes, as the majority of people believe, but also by certain allopathic drugs, such as reserpine, which deplete the granules in the brain that store the neurotransmitters (norepinephrine), serotonin and dopamine. Clinically significant depression is diagnosed in over 15% of people with major depression. Symptoms of major depression include changes in the neurotransmitters monoamine (Norepinephrine) and serotonin (Dopamine)(1). The different mood disorders, depression is the most prevalent. It's a long-term condition that affects your mood, thinking, sleep, appetite, and sex drive. Signs of depression include feeling sad, apathetic, low self-esteem, loss of memory, confusion, and guilt. (2)Depression affects people of all ages, genders and ethnicities. Approximately 121 million people are affected by depression every year. Although there are many drugs available for depression, they all have their limits and there is a pressing need for alternative drugs for mood disorders. Traditional medicine provides many treatment options for depression, most of which are based on plant-based products that have been scientifically tested and proven safe for human consumption in the past. However, medicinal herbs continue to be the preferred treatment for nearly 80 percent of people worldwide, especially in developing countries, to treat and improve the overallhealth. (2).The main reason for this is the widespread belief that plant-derived drugs free from side effects, cost-effective and locally available. India has a long history of using spices in its cuisine. These spices are well-studied in terms of pharmacology and safety. Most of them affect the digestive system, metabolism and the central nervous system (CNS) directly or indirectly via their effects on the neuroendocrine system.

1.2Aetiology of depression:

The aetiology of depression during a person's lifetime cannot be determined by

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biomarkers or anomalies in imaging. The brain's post-mortem examination reveals no systematic abnormalities in structure or neurochemistry. The majority of drugs that are currently on the market were found through empirical means (1). The "amine hypothesis" is the foundation of most modern theories. The primary theory of mood disorders which associated with changes in biogenic amine levels.13–15. It claims that mania due to **excess function** of catecholamine's at the key synapses in the brain, while depression may be due to **functional deficiency** of catecholamine's, especially norepinephrine (NE).Changes in the levels of biogenic amines in the brain, such as norepinephrine (NE), dopamine (DA), epinephrine, serotonin, also known as 5-hydroxytryptamine (5-HT), and two catecholamine's, are thought to be associated with the onset and development of depression (4).

1.3 TYPES OF DEPRESSION THERAPY

1.3.1 Cognitive behavioural therapy (CBT)

The main focus of CBT is on how a person's ideas can impact their regular feelings and behaviours. It is predicated on the idea that unfavourable thought patterns can alter one's behaviour and beliefs, creating a vicious cycle that is challenging to recognise or escape. A person receiving CBT may attend sessions every one to two weeks for a total of five to twenty sessions.

A psychologist or psychiatrist will collaborate with a person throughout a session to analyse their thought patterns and to understand how these patterns may relate to self-destructive behaviours. Numerous unfavourable ideas can occur to someone who is depressed. They can believe that there is nothing they can accomplish well or believe that making an effort to fix a situation is useless.

1.3.2 Interpersonal therapy:

The foundation of IPT is the idea that a person's present interactions and circumstances might affect their mental health. This method is distinct from other forms of treatment that could concentrate on a person's past. It has an organised format and could run 12 to 16 weeks.

i. **A source of pain in a relationship is trouble or conflict:**Arguments with family members, a boss at work, or a tough neighbour can all cause conflict. IPT attempts to assist a person comprehend what they and the other person desire and to improve communication skills to aid in the resolution of continuing problems**.**
ii. **Changes in life** such as the birth of a child or a change in job, can alter a person's perception of themselves and their connections with others. IPT strives to assist people recognize how these changes affect them, as well as gain skills and obtain support to help them cope.
iii. **Grief:** When a person loses a loved one, they may experience depression symptoms. IPT seeks to assist a person in processing their loss by encouraging them to engage in activities and healthy connections with others in order to support them.

Fig.1 grief of depression

Loneliness and isolation: Loneliness and isolation can make a person feel lonely and isolated, making it hard for them to build and sustain meaningful relationships with others. The goal of IPT is to help guide you through the process of building and sustaining meaningful relationships with people.

1.3.3 Psychoanalytical therapy: Psychodynamic therapy aims to help a person learn how their past

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behaviour, which often starts in childhood, relates to their present behaviour. The therapist invites the patient to talk about anything that comes to mind so that they can become conscious of any patterns that are causing their problems and how to address them.

2.1 PLANT PROFILE

Limoniaacidissima Linn (Katha): Limoniaacidissima, commonly referred to as "wood apple," "elephant apple," or "Katha," is recognized as the "poor man's food." Indigenous to regions such as India, Sri Lanka, Penang Island, and Southeast Asian countries like Thailand, Malaysia, Indonesia, and Vietnam, it is also known as "curd apple." Unlike fruits with dedicated plantations, Limoniaacidissima is typically cultivated near riverbanks, roadsides, and fields. Thriving during the monsoon season, this fruit is harvested between March and May, showcasing its seasonal nature. (3)

Wood apple fruits, cultivated in forest areas, boast therapeutic and functional properties across all their parts. The leaves exhibit diuretic and antimicrobial activities while aiding in preventing stomach disorders in children. The roots and bark showcase insecticidal properties and find application in the treatment of snakebites. (4).The fruit pulp is rich in beneficial components such as polyphenols, amino acids, polysaccharides, flavonoids, tannins, antioxidants, and saponins. Additionally, the fruit shell displays activities such as antimitotic and anticancer properties, contributing to skin health by enhancing color complexion. The multifaceted properties of Limoniaacidissima have made it historically valuable in addressing a diverse range of health issues. (9).

2.2 TAXONOMICAL CLASSIFICATION

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliospida
Order: Spindales
Family: Rutaceae
Genus: Limonia L.
Species: L. acidissima

2.3 VERNACULAR NAMES

English: Wood Apple, Elephant Apple, Monkey Fruit or Curd Fruit
Urdu: Kaitha
Hindi: Kath Bel or Kabeet
Oriya: Kaitha
Sanskrit: Kapittha or Dadhistha
Telugu: Vellagapandu
Tamil: Vilampalam

2.4 GEOGRAPHICAL DISTRIBUTION

Kaitha plant has been grown in several tropical countries worldwide such as Africa, Australia, srilanka, malesia, java, Pepang. In India it is cultivated in Karnataka, Delhi, Rajasthan, Orissa, Tamilnadu and Chhattisgarh.

2.5 BOTANICAL DISCRIPTION OF PLANT

Leaves: The leaves of Limoniaacidissima are deciduous, arranged alternately, and measurebetween 3 to 5 inches in length. They are characterized by their dark green color, leathery texture, and often display fine teeth along the edges. The leaf tips are either blunt or notched, dotted with oil glands, and emit a mild lemon fragrance whencrushed. The flowers are small and abundant, typically appearing in dull red or greenish hues. They are arranged in small, loose clusters forming terminal or lateral panicles. (3).

Fruits: The fruit is round to oval, globose, large, with a diameter of 2 to 5 inches. The skin is hard and woody, with a greyish white to blackish scabbing, with a thickness of about 6 mm. (11).

Flowers: flowers are normally bisexual.(3).

Seeds: The seeds were brown in colour, oblong elliptical in shape and contains two cotyledons which were plano convex.

Shell: Wood Apples look like tiny coconuts, with a diameter of 5 to 12 centimetres. The shell is hard, woody and white to light brown in color, with a rough texture similar to tree bark. (3)

2.6 TRADITIONAL USES:

- Dysentery
- Diarrhea
- Asthma
- Wounds
- Tumors
- Hepatitis
- Liver disease
- Heart disease

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- Blood purification
- Progesterone deficiency
- Breast and uterus cancer
- Infertility
- Menorrhagia

3.MATERIALS AND METHODS:

3.1 collection of the sample material:

The fruits of Limoniaacidissimalinn was collected from the fruit market which was nearer to JNTUH kukatpally, in the month of August 2022.

Fig.4.1Raw Limoniaacidissima

3.2 IDENTIFICATION AND AUTHENTICATION

Dr. A. Vijay Bhaskar Reddy, Assistant Professor, Department of Botany, University College of Science, Osmania University, Hyderabad, verified and taxonomically identified the fruit. Osmania University preserved the identified specimen.

3.3 PROCESSING OF PLANT MATERIAL:

3.3.1 DRYING AND PULVERISING:

The pulp of unripe fruit was extracted by breaking its shell. After cleaning, the shell is left out in the sun to dry. Using a pulveriser (a device that grinds materials mechanically), the dried material was ground into powder. The powder was then weighed using an electronic balance, and the ground material was stored for next use.

3.3.2 DETERMINATION OF EXTRACTIVEVALUES:

5 g of powdered Limoniaacidissima was precisely measured and transferred into a 250 ml conical flask and macerated with 100 ml of each of the 4 solvents (hexane, ethyl acetate, methanol, and ethanol)for 2 days, shaking constantly every 3 hours, while covering it with foil then transfer 25 ml of each solvent through a filter into a thin porcelain dish that had previously weighed. Calculate the % weight to-weight of the extractive values for each solvent after evaporating to dryness using the formula.

Extractive value = W1- W2 / Weight of plant materialtaken X 100

Where,

W1= weight of the china dish without extract

W2= weight of the china dish with plant extract

3.3.3 EXTRACTION OF PLANT MATERIAL:

SOXHELT EXTRACTION: In this procedure, approximately 40g of previously air-dried and coarsely pulverized Limoniaacidissimalinn powder serves as the sample, placed within a thimble. One-fourth of the round bottom flask (RBF) is filled with ethanol, and porcelain chips are introduced to prevent bumping. The entire apparatus is assembled, with a heating mantle employed to heat the RBF. As the heating progresses, the siphon initiates overflow due to gravity, and the spilled liquid returns to the RBF through direct linkage. This marks the commencement of the first cycle. Subsequently, the product collected in the round bottom flask is processed. The process described can be iterated numerous times over the course of hours or days until the colour of the solvent diminishes in the siphon tube. Subsequently, the solvent is drawn back into the round bottom flask for the evaporation process.

Fig 6 soxhelation process

3.3.4 EVAPORATION:

The ethanol extract was stored in a rotational evaporator. Rotational evaporators work on the principle that the boiling point of solvents decreases with decreasing pressure. The evaporator flask spins at a specific speed, causing the material to form a thick film on the inner side of the flask.Then the product was transferred into the porcelain dish and covered with the aluminum foil.

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Now the product had become ready for experimental use.

% yield = weight of the obtained dried extract / weight of the plant materialX 100.

Fig.6 Recovery of the solvent by Rota Evaporator

3.4 EXPERIMENTAL ANIMALS:

The animals were kept in regular temperatures, humidity levels, and 12-hour cycles of light and darkness. They were kept in polypropylene cages, fed a typical pellet diet, and given unrestricted access to water. The study's procedures and experiments were approved by the institutional animal ethics committee of PRIP College of Pharmacy in Hyderabad.

3.5 EXPERIMENTAL DESIGN:

For assessing the anti-depressant activity, there are two in vivo models:

1. Forced swimming test
2. Tail suspension test

PRINCIPLE:Imipramine is effective drug in the management of depression .It acts by blocking dopamine receptors which is responsible for mood, temperature, emotions, appetite and behaviour. With the help of Forced swimming test and Tail suspensions the antidepressant activity of drug within the duration of time can be determined. The study was conducted according to the reported method of (Steru et al., 1985)."

3.5.1 FORCED SWIMMING TEST

ANIMAL GROUPING:

Group	Treatment	Dose	No. of animals
G_1	**Control**	2ml/kg	**4**
G_2	**Standard**	10mg/kg	**4**
G_3	**Low drug extract**	(50mg/kg)	**4**
G_4	**High drug extract**	(200mg/kg)	**4**

PROCEDURE:

"Adult mice were randomly divided into 4 groups of 6each."

Group-1:-Received 2ml/kg body weight I.P of normal saline.

Group-2:-Received 10mg/kg body weight standard imipramine drug I.P respectively.

Group- 3:-Received Ethanol extract of plant drug at dose of 50 mg/kg,body weight I.P.

Group-4:- Received Ethanol extract of plant drug at dose of 200 mg/kg,body weight I.P.

Test should be performed in an open cylindrical container (10*25)cm containing 19 cm of water at 25±1◦C.

After 2 minutes animal shows the vigorous movements,then after 4 minutes manually record the immobility of animals.

After 6 minutes remove the animal and towel dry it and resend to cage.

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Fig. 6 experimental behaviour of mice in forced swimming test

3.5.2 TAIL SUSPENSION TEST:

ANIMAL GROUPING:

Group	Treatment	Dose	No. of animals
G_1	**Control**	10ml/kg	**4**
G_2	**Standard**	4mg/kg	**4**
G_3	**Low drug extract**	(50mg/kg)	**4**
G_4	**High drug extract**	(200mg/kg)	**4**

PROCEDURE:

The study conducted according to the method of steru et al.,(1985).

Adult mice were selected for the procedure.

Group-1:- received normal saline 10ml/kg.

Group-2:- received 4mg/kg imipramine I.P respectively.

Group -3:- received graded doses of ethanolic extract ofLimoniaacidissima(unripe fruit rind extract) about 50mg/kg.

Group-4 :-200mg/kg body weight I.P.

After 30 minutes of I.P treatment each mice was suspended by tail on the edge of a shelve 58cm above a table top and length, immobility time should be recorded for 6 minutes."

Mice were considered immobile when hung passively and remain motionless.

Fig.8 Tail suspension test

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II. RESULTS AND DISCUSSION:

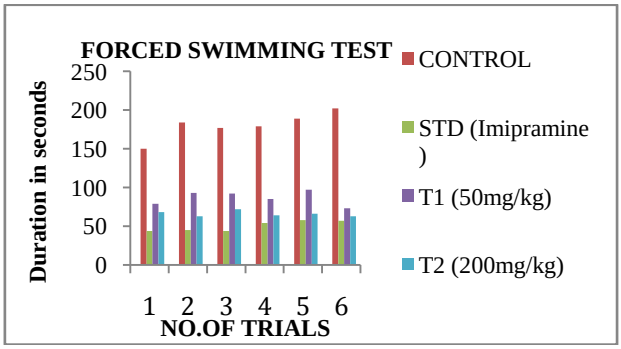
IN-VIVO ANTIDEPRESSANT ACTIVITY:

Ethanolic unripe fruit rind extract of limoniaacidissima was explored for antidepressant activity in mice model. All the results obtained from the experiment were represented below:

EFFECT OF EELA ON FORCED SWIMMING TEST(FST):

S.No.	Groups	Treatment and Dose	Duration of Immobility in seconds
1	Normal Control	Distilled water 10ml/kg	180.16 ± 0.0185****
2	Standard	Imipramine(10 mg/kg)	53.5 ±0.5****
3	Test-1	Ethanolic unripe fruit rind extract of Limoniaacidissima (50mg/kg)	71 ± 0.3
4	Test-2	Ethanolic unripe fruit rind extract of Limoniaacidissima(200 mg/kg)	86.5 ± 0.1**

Statistical comparison: One way ANOVA, followed by Tukey's multiple comparison test. Control, Standard, EELA (50 mg/kg) and EELA (200 mg/kg) are compared within them PP=(p<0.0001).**



EFFECT OF EELA ON TAIL SUSPENSION TEST(TST):

S.No.		Groups	Treatment and Dose	Duration of Immobility in seconds
1		Normal Control	Distilled water 2ml/kg	116.16 ± 0.0185****

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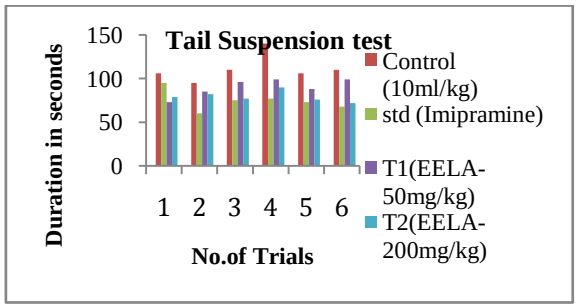
2		Test-1	Ethanolic unripe fruit rind extract of Limoniaacidissima (50mg/kg)	71 ± 0.3
3		Test-2	Ethanolic unripe fruit rind extract of Limoniaacidissima(200 mg/kg)	79.3± 0.1**
4		Standard	Imipramine (4mg/kg)	93.66 ±0.5****

Effect on the time that mice hanging by their tails remained immobile (≤ p 0.001). In comparison to the control, imipramine employed as a positive control considerably (≤ p 0.001) decreased the length of immobility in mice.



III. DISCUSSION:

Limoniaacidissimaalso known as wood apple .Several ethnobotanical applications of Limoniaacidissima (L.) have been documented, includes the usage of the fruit as a sour, sweet, refrigerant, cardio tonic, liver and lung tonic, astringent, and beneficial against dysentery, diarrhea, throat infections, and gum illnesses. The fragrant, carminative leaves are useful for treating diarrhoea and vomiting cough. Heart ailments and migraines are cured by seeds. Sometimes a bark is given for biliousness. (9)

The fruits of Limoniaacidissimalinn was collected in the month of august and authenticated. After cleaning, the shell is left out in the sun to dry. Using a pulveriser the dried material was ground into powder. The four solvents were used to determine the extractive values (hexane, ethyl acetate, methanol, and ethanol), ethanol was found to have more extracted values when compared to other solvents. Hence ethanol was considered as suitable solvent for extraction of the phytoconstituents from the plant product.

Phytochemical screening of Limoniaacidissima (L) leaves, bark, fruit pulp, and fruit rind reveals the presence of several compounds such as carbohydrates, proteins, amino acids, tannins, alkaloids, steroids, saponinand glycoside. (7)

The conventional usage of these plants to treat a variety of ailments may be somewhat justified by the presence of secondary metabolites. However, steroidal glycoside that has been separated from plants is well-known for having cardio tonic qualities.

The antidepressant activity can be observed in ethanolic unripe fruit rind extract which was evaluated by the two models FST and TST by using albino mice. The ethanolic extract of Limoniaacidissimalinnshowed great potential by observing the behavioural changes in mice. when compare to the control, test drug showing the immobility in mice at 50mg/kg of EELA, and the significant effect observed at 200mg/kg.

The extraction method was done by soxhelt apparatus and the percentage yield was found to be 24.03%.The unripe fruit rind extract of Limoniaacidissima was found to be safe up to 2000 mg/kg bd. wt.

IV. SUMMARY:

Due to their diverse properties, herbs play a crucial role in various industries in today's world. LimoniaAcidissima., commonly known as Wood

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Apple, belongs to the Rutaceae family. Different parts of LimoniaAcidissima.exhibit distinct properties, encompassing saponins, flavonoids, amino acids, beta-carotene, tannins, carbohydrates, vitamin B, and triterpenes. The available information underscores the unique pharmacotherapeutic properties of LimoniaAcidissima. Traditionally, it has been utilized to address a range of human ailments, including dysentery, cardiac problems, liver issues, sore throat, and exhibiting antibacterial, antioxidant, anti-diabetic, and wound-healing properties."

For the evaluation of antidepressant activity unripe fruit rind extract was collected, authenticated and extractive values were determined.

"Depression stands as one of the most prevalent psychiatric disorders, and the World Health Organization (WHO) anticipates a continued increase in its prevalence. To evaluate the antidepressant potential, immobility time was measured using the Tail Suspension Test and the Forced Swim Test. The ethanolic extract of Limoniaacidissima Linn demonstrated significant promise, as evidenced by observable behavioural changes in mice. In comparison to the control group, the immobility time notably decreased in mice treated with ethanolic unripe fruit rind extracts (Limoniaacidissima - 50 mg/kg and Limoniaacidissima - 200 mg/kg). Importantly, the extract exhibited safety up to a dose of 2000 mg/kg body weight. These findings suggest a potential therapeutic role for Limoniaacidissima in addressing depressive symptoms."

CONCLUSION:

The present study suggest that ethanolic extract of unripe fruit rind of Limoniaacidissima provides significant protection against depression (mental disorder). Immobility time was measured in the Tail suspension test and the forced swim test to assess antidepressant activity. Immobility time was reduced in the standard group when compared to the control group. Therefore ethanolic extract of unripe fruit rind extract can be used in the management of mental disorder.

FUTURE SCOPE:

The underutilized fruit, Limoniaacidissima, commonly known as wood apple, emerges as a valuable resource with nutritional, therapeutic, and curative potential. This fruit is rich in phytochemicals such as polyphenols, coumarins, phytosterols, saponins, tannins, flavonoids, and flavonols. Additionally, it contains essential vitamins like thiamine, riboflavin, niacin, vitamin C, along with minerals and amino acids, contributing to its antioxidative and antifungal properties. The diverse array of bioactive compounds in Limoniaacidissima underscores its potential significance in promoting health and well-being."

This research will be helpful to create interest towards lesser known facts about wood apple. Also it has less or no documented form on antidepressant activity.

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