PHARMACOLOGICAL EVALUATION OF INVITRO ANTIOXIDANT AND INVIVO ANTIDEPRESSANT ACTIVITIES OF ETHANOLIC BARK EXTRACT OF *ANNONA MURICATA* ON MICE

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Submitted by

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CERTIFICATE

This is to certify that the research work entitled **"PHARMACOLOGICAL EVALUATION OF INVITRO ANTIOXIDANT AND INVIVO ANTIDEPRESSANT ACTIVITIES OF ETHANOLIC BARK EXTRACT OF ANNONA MURICATA ON MICE"** is a bonafide research work done by **Mr.T.T.J.S.SRINIVASAGUPTHA** (Regd. No.: 623269025016) in the Department of Pharmacology under my guidance and supervision. I recommend for submission of the project dissertation to Andhra University, Visakhapatnam in partial fulfillment of the requirement for the award of the degree of Master of Pharmacy in Pharmacology.

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BONAFIDE CERTIFICATE

This is to certify that the research work entitled **"PHARMACOLOGICAL EVALUATION OF INVITRO ANTIOXIDANT AND INVIVO ANTIDEPRESSANT ACTIVITIES OF ETHANOLIC BARK EXTRACT OF ANNONA MURICATA ON MICE"** submitted by student **Mr.T.T.J.S.SRINIVASAGUPTHA** (Reg. No. 623269025016) in the Department of Pharmacology under the guidance and supervision of **Mrs.K.V.NAGA LAKSHMI.,M.Pharm.,(Ph.D).,** Associate Professor in department of Pharmacology, VJ's College of Pharmacy being duly certified and approved for submission of the project dissertation to Andhra University, Visakhapatnam in partial fulfillment of the requirement for the award of the degree of Master of Pharmacy in Pharmacology.

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DECLARATION

I hereby declare that this dissertation entitled **"PHARMACOLOGICAL EVALUATION OF INVITRO ANTIOXIDANT AND INVIVO ANTIDEPRESSANT ACTIVITIES OF ETHANOLIC BARK EXTRACT OF ANNONA MURICATA ON MICE"** has been carried out under the supervision and guidance of **Mrs.K.V.NAGA LAKSHMI.,M.Pharm.,(Ph.D).,** Associate Professor in the Department of Pharmacology, VJ's College of Pharmacy, Diwancheruvu, Rajamahendravaram during the academic year 2025 and submitted to Andhra University in the partial fulfillment of the requirement for the award of degree of Master of Pharmacy in department of Pharmacology.

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Date: **(T.T.J.S.SRINIVASAGUPTHA)**

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LIST OF ABBREVATIONS

A.muricata	*Annona muricata*
SNRIs	Serotonin Nor-epinephrine Reuptake Inhibitors
TCAs	Tricyclic anti-depressants
OB	Olfactory Bulbectomy
ROS	Reactive oxygen species
FST	Forced Swim Test
TST	Tail Suspension Test
DPT	Digital Photoactometer Test
OFT	Open Field Test

ABSTRACT

1. ABSTRACT

The present investigation aims to elucidate the pharmacological potential of the ethanolic bark extract of *Annona muricata* L. (Annonaceae) with respect to its in-vitro antioxidant and in-vivo antidepressant activities. Preliminary phytochemical analysis of the extract revealed the presence of flavonoids, phenolic compounds, alkaloids, and saponins, which are known to exhibit neuroprotective and free-radical scavenging effects. The in-vitro antioxidant potential was evaluated by the Phosphomolybdenum assay, where the extract demonstrated a concentration-dependent increase in total antioxidant capacity, comparable to that of the standard antioxidant ascorbic acid. The in-vivo antidepressant effect was assessed using validated behavioral paradigms including the Forced Swim Test (FST), Tail Suspension Test (TST), Open Field Test (OFT), and Photoactometer assay in albino mice. The extract at doses of 75 mg/kg and 150 mg/kg p.o. produced a significant reduction in immobility time and an increase in locomotor activity, indicating antidepressant-like activity comparable to the standard drug imipramine. The findings suggest that the ethanolic bark extract of *A. muricata* possesses potent antioxidant and antidepressant properties, possibly mediated through monoaminergic modulation and attenuation of oxidative stress, thereby supporting its ethnopharmacological relevance and potential as a natural therapeutic candidate for depressive disorders.

Keywords

1. *Annona muricata* L.
2. Antioxidant potential
3. Antidepressant activity
4. Phytochemical analysis
5. Behavioral models (FST, TST, OFT)

AIM AND OBJECTIVES

2. AIM AND OBJECTIVES

AIM:

The main aim of present work is to Pharmacological Evaluation of Anti-oxidant and Anti-depressant activities of ethanolic bark extracts of *Annona muricata* on mice which is not reported in past. So a sincere effort has been made to evaluate these biological activities.

OBJECTIVES:

Based on the background information available about the plant, the present work has been divided into 3 main objectives:

1. To prepare ethanolic bark extract of *Annona muricata* and perform its preliminary phytochemical analysis.
2. To evaluate in-vitro Anti-oxidant activity of ethanolic bark extract of *Annona muricata* using

- Phosphomolybdenum Antioxidant assay.

3. To evaluate in-vivo Antidepressant activity of ethanolic bark extract of *Annona muricata* using

- Forced swim test(FST)
- Tail suspension test(TST)
- Locomotor activity using photoactometer
- Open field apparatus test.(OFT)

INTRODUCTION

3. INTRODUCTION

Depression is a common factor which is mostly experienced by everyone at one time or another. Symptoms may vary depending on the person. Many people fall down occasionally, feeling drowsy, lack of social behavior, feeling tensed and many more consequences have been observed. According to WHO survey report, 350 million people are suffering from mental disorders especially of depression.

Depression is a whole body illness which involves mood and emotional fluctuations resulting in hopelessness, sadness, changes in sleep, loss of appetite and suicidal thoughts and is predicted to be the second largest source of any mental disorders in the upcoming years. Two types of mental depression have been noticed namely unipolar and bipolar.75%cases are mostly noticed due to unipolar depression and 25%cases are noticed under bipolar depression which is also known as endogenous depression which results in mania over a period of a few weeks. Since, it is a heterogeneous disorder; it could be treated in various methods depending on the symptoms of person.

Many synthetic drugs are acting as the standard treatment clinically for depressed patients, where they reflect in many adverse effects which clinically compromise the actual therapeutic treatment. Some common side effects include gastrointestinal disorders along with respiratory problems, anxiety, fatigue, dry mouth, mainly cardiac arrhythmias. Many drug interactions will be taking place which immediately require an alternative therapy without any side effects and good recovery which is using a medicinal plant for better results.

A number of medicinal plants and medicine derived from those plants are showing antidepressant properties with proved benefits and minute risk ratio.Plants are being used for medicinal uses since a long ago. According to Unani manuscripts and Chinese writings in past they elevated the importance of herbs and their medicinal benefits. Over 4000 years Indian Vaids, Mediterranean cultures and European countries are using herbs as medicine. After healing rituals of herbs they are been used by many countries later and traditional medical systems such as Ayurveda, Unani and Chinese medicine systems are developed as systemic therapies.

Pharmacotherapy along with psychotherapy is mostly prescribed treatments for people suffering from depression because patients with depression will be having symptoms which

reflects the decrease in monoamine neurotransmitters especially serotonin, norepinephrine and dopamine in brain. Such that those drugs can influence the altered neurotransmitters and can reduce depression. So it is given that anti-depressant drugs to a patient are given based on the type of altered neurotransmitter. Tricyclic anti-depressants, serotonin reuptake inhibitors, nor-epinephrine reuptake inhibitors,serotonin-nor-epinephrine reuptake inhibitors and dopamine reuptake inhibitors are used as the pharmaceutical drugs for treatment of depression (Biokomo E.O. et al., 2017). The work is based on the medicine derived from a medicinal plant and evaluating its benefits regarding the treatment of depression which could be useful for further investigations.

Antioxidants scavenge free radicals and produce resistance against oxidative stress, and also prevent diseases by inhibiting lipid peroxidation. At present there is an interest elevated worldwide to recognize pharmacologically effective anti-oxidant elements that are potent withside effects nil, after their use in food industry,preventive medicine etc. Plants provide required antioxidants which ceasethe oxidative stress caused by oxygen and photons;which appear to be a potential source of new compounds with antioxidant activity. Important health care of India is the traditional herbal medicine. Ayurveda is intended to be the elderly medicine system in the world which provides potential lead to find therapeutically active and purposeful components from plants.

DEPRESSION

Depression is found to be a state of low mood and extremely common psychiatric condition which shows aversion from activities that an affect thoughts, feelings, behavior, and sense of well-being for a person. It may start slowly ranging from a mild condition followed by bordering normally to severe depression associated with delusions and hallucinations.

People suffering from depression might be sad or anxious suddenly which leads to mood disorders this may cause the person feel hopeless and helpless some may also have guilty, irritability, anger that effect a person's personal and physical behavior.Many people fall down occasionally, feeling drowsy, lack of social behavior, feeling tensed and many more consequences have been observed. According to WHO survey report, 350 million people are suffering from mental disorders especially of depression this condition may increase further in upcoming years (Biokomo E.O. et al., 2017).

Symptoms:

The persistent feeling of sadness or loss of interest in social activities may vary characteristically and cause depression which might lead to a range of physical and behavioral symptoms. Depression may also be linked with the ideas of suicide.

Emotions Based:

Apathy, Anxiety, General discontent,Hopelessness, Guilt,Loss of interest and pleasure in activities Sadness or Mood swings,Lack of concentration, Low self-esteem,No motivation Thoughts of death and suicide.

Behavior Based:

Excessive crying, Agitation, Irritability Social isolation orRestlessness Slowness in activity, Low concentration, Waking up early, Excess sleepiness, Restless sleep or Insomnia.

Biological thoughts include:

Retardation of thought and action Sleep disturbances and loss of appetite.

4.1 TYPES OF DEPRESSIVE SYNDROME:

Regarding the clinical classification of two types of mental depression have been noticed namely unipolar and bipolar.

Unipolar depression: Very common condition and could be treatable. Changes in neural circuit activity might be a cause. Mood swings are always in the same direction.

It is a condition of mental illness or melancholia, intense sadness or despair that has advanced to the point of being disruptive to an individual's social functioning daily life activities. It is also known as a major depressive disorder

Major cases of this unipolar depression are commonly unfamiliar which is strictly associated with stressful life accompanied with anxiety and agitation which is also termed as reactive depression which effects the education, eating habits and personal life etc.

Very few cases are familiar which are unrelated to external stress and with different symptoms. Even 50% of the people suffering from depression or another mood disorder die by suicide (Bachmann, S 2018).

Bipolar depression: Bipolar depression is difficult to be diagnosed, because it looks similar to depression when someone is in a low stage, a person can also experience depressive episodes for several years withoutmentioning the experience of mania or hypomania. Bipolar depression is commonly known as 'manic depression' because a person experiences several episodes of depression and mania, during this the person may have poor eye contact, negative outlook on life they may harm themselves and may also develop anxiety disorders (Anderson IM etal., 2012).

4.2 THEORIES OF DEPRESSION:

According to a survey it is said that neurochemical imbalance is the pathophysiology for mood disorders. The most widely accepted theory for depression is monoamine hypothesis.

Monoamine hypothesis: States that the underactivity of monoamines and especially 5-HT (5-hydroxy tryptamine) is the major cause of depression. It is associated with a systemic deflect of monoamine transmitters at certain sites in the brain. This theory has been evolved from the success of drugs that elevate the concentration of serotonin mainly neurotransmitters in the

synaptic clefts throughout the brain. Yet despite its popularity many observations and findings challenge this theory.

Compounds those deplete the serotonin, monoamine levels in brain may not cause any depression in normal healthy people while the same change may cause depression in functionally defective patients (Delgado PL.2000).

4.3 BIOCHEMICAL EVIDENCE:

Biochemical studies do not exactly support the monoamine hypothesis in its simpler formon depressed patients. The major metabolites for nor adrenaline and 5-HT respectively are 3-methoxy -4hydroxy phenyl glycol (MPHPG) and 5-hydroxy indoleacetic acid (5-HIAA).

Those are present in the blood, urine andcerebrospinal fluid.Urinary MHPG excretion studies in depressed andnormal subjects have convincingly showed that the level is decreased in depressed ones. Depressed patients are known to have higher Plasma noradrenaline levels than normal people.

4.4 NEUROPLASTICITY AND TROPHIC EFFECTS:

Fundamental mechanism of neuronal adaptation is the neural plasticity, which could be disrupted in depression. The changes might occur in neural plasticity due to stress or other negative stimuli which plays a prominent role in the development and onset of depression (Wei Liu, et al., 2017).

Antidepressant treatments have found to exert their antidepressant effects on neural plasticity. However complete mechanism of neural plasticity still remains mysterious, it is associated with neuronal loss and decreased neurogenesis in the hippocampus and prefrontal cortex

4.5 PRESENT TREATMENTS OF DEPRESSION

Pharmacotherapy along with psychotherapy is mostly prescribed treatments for people suffering from depression, such that those drugs can influence the altered neurotransmitters and can reduce depression. So it is given that anti-depressant drugs to a patient are given based on the type of altered neurotransmitter some of those drugs are

Tricyclic anti-depressants, Serotonin reuptake inhibitors, Dopamine reuptake inhibitors,

Nor-epinephrine reuptake inhibitors (National Collaborating center for mental health (UK)).

Reuptake inhibitors of Serotonin-nor-epinephrine are used as the pharmaceutical drugs for treatment of depression

4.6.1 Tricyclic antidepressants:

These are been discovered in 1950's which contains three rings of atoms and were named after their chemical structure. These are utilized as first line drugs for treating depression. They primarily act as SNRIs by blocking the serotonin transporter and the nor-epinephrine transporter,those resultsin the elevation ofconcentrations of the neurotransmitters and finally enhancement of neurotransmission. Both serotonin and norepinephrine are also indicated in depression and anxiety while falicitation of their activity has beneficial effects on these mental disorders. TCAs include imipramine, nortriptyline, protriptyline, desipramineetc ((National Collaborating center for mental health (UK)).

Side effects include: constipation, blurred visionand dry mouth, weigh gaining, weight loss, low blood pressure on standing, rashes, increased heart rate.

4.6.2 Serotonin reuptake inhibitors:

These are the antidepressants which were mostly prescribed which cause fewer side effects. They work by elevating serotonin levels in the brain which reduces depression, themost important neurotransmitters in brain like serotonin are also known as a chemical messenger that mediates signals from cells of brain. SSRIs act bymaking more serotonin available near the sites and thus, block the reabsorption of serotonin in the brain (Presynaptic neuron). SSRIs seem to primarily affect serotonin, not other neurotransmitters so are called selective (National Collaborating center for mental health (UK)).SSRIs include Escitalopram,Fluoxetineand Citalopramетc.

Side effects include: insomnia, diarrhea, drowsiness, nausea and dry mouth.

4.6.3 Nor epinephrine reuptake inhibitors:

Adrenergic reuptake inhibitor is also known as a Norepinephrine reuptake inhibitor.These belong to the class of drugs which acts as a reuptake inhibitor for the neurotransmitters like epinephrine and norepinephrine by blocking the transporter action of norepinephrine. This in turn leads to elevated extracellular levels of epinephrine and norepinephrine and thus adrenergic neurotransmission increases.

These were mostly used in treating conditions such as narcolepsy, due to their psychostimulant effects.These are also used as an antidepressant in the treating major depressive disorder.

These are some selective NRIs: Amedalin, Atomoxetine, Daledalin, Lortalamine, Nisoxetine, Talopram, Talsupram, Tandamine

Side effects include: drymouth, blurred vision, drowsiness, excessive sweating, insomnia, constiparion, headache, dizziness, urinary retention.

4.6.4 Dopamine reuptake inhibitors:

They act as a reuptake inhibitor by blocking the action of dopamine transporter, when extracellular dopamine is not absorbed by the postsynaptic neuron,then reuptake inhibiton takes place. So, Re-entryof the presynaptic neuron is blocked. This results in dopaminergic neurotransmission due to increased extracellular concentrations of dopamine. These were mostly used in treating narcolepsy and also as psychostimulants, obesity, eating disorders. These are occasionally used as antidepressants especially in treating mood disorders.

Few of those DRIs are: Altropane, Amfonelic acid, Amineptine, Armodafinil.

4.6.5 Selective serotonin and noradrenaline re-uptake inhibitors(SNRIs):

Reduced levels of the monoamines in the brain, such as 5-HTis mostly associated with depression. These selective reuptake inhibitors ofnoradrenaline and 5-HT (SNRIs) were assumed to restore the concentrations of noradrenaline and 5-HT in the synaptic cleft by binding at their re-uptake transporters avoiding the reuptake and subsequent degradation of noradrenaline 5-HT (National Collaborating center for mental health (UK)).

This reuptake results the monoamines to accumulate in the synaptic cleft and thus, normal range of concentration is found. The SNRIs action is through to contribute to the elevation of the depression symptoms. Small amounts of noradrenaline and 5-HT continue to be degraded in synaptic cleft, by the presence of SNRIs.

Some of these SNRIs are: Duloxetine, Venlafaxine,Desvenlafaxine, Levomilnacipranetc.

Side effects include: Dizziness, nausea, dry mouth, sweting, tiredness, insomnia, anxiety, constipation.

4.6.6 Mono amine oxidase inhibitors (MAOIS)

Those drugs which decrease the activity of mono amine oxidase enzymes are known as mono amine oxidase inhibitors. Mono amine oxidase inhibitors i.e., monoamine oxidase enzyme A (MAO-A) and mono amine oxidase enzyme B (MAO-B). Since these are having a very long history in treating depression these are most effective treatment for atypical depression((National Collaborating center for mental health (UK)).

Drugs under MAOIs include: phenelezine, selegiline, isocarboxazid

4.6 PSYCHOTHERAPY:

The word psychotherapy has been evolved from the ancient Greek word psyche and therapeia, which means it is the treatment of disorders related to personality, mind or by psychological methods. Psychotherapy is the intentional application of interpersonal stances and clinical methodswhich are derived from established principles of psychologyto change theircognitions, behaviors, emotions and other personal characteristics in directions that the participants in directions that the participants deem desirable. Commonly used psychotherapy treatments are inter personal therapy (IPT), cognitive behavioral therapy (CBT), electro convulsive therapy (ECT).

4.6.1 Cognitive behavioural therapy (CBT)

This CBT is anmanualized, short term, present oriented psychotherapy that has demonstrated robust and replicable results, as both an acute and maintainance treatment for residual symptoms and depression. Acute cognititve therapy involves typically 15 to 20 weekly sessions for 50 minutes. CBT is as effective as anti-depressant drugs which reduce the symptoms and the effect remains upto one year after the treatment ends. CBT along with anti-depressant drugs gives effective results compared to anti-depressant drugs alone.

4.6.2 Interpersonal therapy:

Interpersonal psychotherapy is an empirically supported treatment and it is a short and a manualized psychotherapy which shows the reliable results. Acute interpersonal therapy involves 3 phases over 12 to 16weekly sessions.

It is an observation legalized intercession for depressive disorders, and is mostly potent when given in combination with psychiatric medications. Cognitive behavioural therapy, interpersonal therapyand electroconvulsive therapy are the only psychological interventions and treatment of choice for psychiatry residents.

4.6.3 Electro convulsive therapy:

There are other options to try, when medication fails to ease the symptoms of clinical depression. Electroconvulsive therapy is a brain stimulation techniqueused to treat major depression. This therapy is used when all the other treatments fail to work.

4.6.4 Future antidepressant drugs:

Many different methods are being used, and many compounds are under evolution. Those involve neuropeptide antagonist like substance –P, CERI and also compounds that are active on acetylcholine, histamine and NMDA receptors and also on compounds those act upon signal transduction pathways that are responsible for apoptosis, neuroplasticity and neurogenesis.

To satisfy the following criteria is the main aim:

- Low side effects
- Lower toxicity in overdose Rapid action
- Grater efficacy
- Efficacy in patients not responsive to MAOIsor TCAs .

However, many compounds have showed efficiency in clinical trials, none appear better than existing drugs with respective it these criteria.

4.6.5 Behavioral and Pharmacological models for the evaluation of antidepressant activity

Exact etiopathology of depression is unknown. The basic considerations in the field of antidepressants is that tests to the model of depression do not exist. Any type of mentioned models for depression needs to meet and satisfy construct predictive and face validities. Antidepressants exert its actions in both depressed and non depressed subjects. This implies that the use of normal cells and animals only involves pharmacological actions rather than therapeutic actions of drugs.

Table 1.: Behavioral and pharmacological models for the evaluation of antidepressant activity

S.NO	MODEL TYPE	METHOD	REFERENCES
1.	PHARMACOLOGICAL MODELS	• Reversal of reserpine and tetrabenazine • Yohmbin potentiation • 5hydroxytryptophan Potentiation • Potentiation of apomorphine induced aggression	Dana Yaffe, et al., 2018 Willner.P 1884,1991 W.Schreiber, etal., 1999 Maj J, 1979
2.	DIATHESIS MODELS • Lesion models • Genomic models • Genetic models	• Olfactory bulbectomy • HPA system • Fawn hooded	Kelly JP (1997); Barden N (1996); Rezvani AH, et al (2002)
3.	STRESS MODEL	• Tail suspension test • Forced swim test • Learned helplessness • Open field apparatus • Restraint stress	Porsoltetal.,(1977); Steru L etal.,(1985); ML Seibenhener 2015 Martin P etal.,(1989); Kennetetal.,(1987); JooYeongetal., 2013.
4.	SOCIAL DOMINANCE MODELS	• Neonental isolation and adult isolation • Social hierarchy and social defeat	Robrtson J and Bowbly(1952); Reite M (1981); Albonetti ME and Farabollini F (1994).

Pharmacological tests: These tests include pharmacological interaction between different types of drugs.

Reversal of reserpine and tetrabenazine:

Reserpine may cause hypothermia and also irreversibly blocks the vesicular monoamine transporter. On the other hand depletion of these (nor adrenaline, dopamine, 5-hydroxytryptamine) concentrations in brain also can cause hypothermia. This results in the decreased body temperature which is induced by reserpine and is antagonized by antidepressants. For antidepressantsthe reversal of hypothermia is not specific. Amphetamines and some antipsychotic agents (chlorpromazine) can also antagonize the fall in body temperature. Thus metabolic rate of monoamine neurotransmitters are increased by reserpine; and also reduces the magnitude of releasing monoamines. Depletion of dopamine concentrations can also cause drug-induced Parkinsonism .It may take some time or some days to regain the depleted monoamine transporter, hence it shows that reserpine effects are long lasting (Dana Yaffe, etal., 2018).

Tetrabenazine is known to act as a monoamine depleting and dopamine receptor blocking drug. This TBZ is responsible for the depletion of noradrenaline, dopamine, and serotonin without affecting their own synthesis. Tetrabenazine also inhibits vesicular monoamine transporter2, reversibly which results in the reduced uptake of monoamines into synaptic vesicles, depletion of monoamine storage as well (W. Schreiber, etal., 1999).

Yohimbine potentiation:

Through vasodilation and vasoconstriction, yohimbine might decrease or increase the systemic blood pressure. $\alpha_2$receptor's affinity is higher for yohimbine, relatively selective α_2 blockade is done by increase in blood pressure which is caused by giving small doses., However, yohimbine also interacts with receptors of α_1, with low affinity; therefore, at higher doses a α_1 blockade can occur and overwhelm the effects of the α_2 blockade, which leads to a potential dangerous drop in blood pressure. Oral yohimbineat higher doses can create numerous side effects including overstimulation, rapid heart rate, anomalous blood pressure, insomnia and cold sweating.

5-Hydroxyltryptamine potentiation:

In an open trial study, 14 patients suffering from bipolar and unipolar depression are been treated with L-5-Hydroxytryptophan (L-5-HTP) and benzerazide, are also given with L-Deprenilwhich is a selective and irreversible MAO-B inhibitor without 'chesse effect'. Ten in

14 patients exhibitedthe required responses to the combination of drugs and a good relationship was found between the clinical response and degree of platelet MAO inhibition.

In a controlled double-blind study,14 affectively ill patients were allocated randomly toL-5- HTP, L-Deprenil andbenzerazide, 21 patients were treated usingbenzerazideand L-5-HTPand other 19 patients were treated only with placebo. Patients who were given treatment with the combo of L-5-HTP and L-Deprenilshowed significantimprovement clinically compared to patients treated with placebo, but this was not the case for patients who were treated alone with 5-HTP. Patients treated with L-Deprenil exhibited a positive correlation between degree of platelet MAO inhibition and mood improvement.

DIATHESIS MODELS:

The term diathesis has been evolved from the Greek word (διάθεσις) for sensibility or predisposition, which means it is a theory of psychologythat seeks to explain a disorder, or its course, as the result of an interaction between stress and predispositional vulnerability occurred through life experiences.

Olfactory Bulbectomy Model:

The proposed animal model of depressionis olfactory bulbectomized (OB) rat. The below mentioned behavioral changes are been noticed following bilateral olfactory bulbectomy: hyperactive in an enclosed area, such as the open-field; in a 24-hr home cage activity monitor increased nocturnal hyperactivity is recorded; memory deflect, when observed using 8-arm radial maze,Morris maze and passive avoidance behavior; more open arm entries in elevated plus-maze; and differences in conditioned taste aversion behaviorfood motivated. Alterations in the serotonergic, glutamatergic neurotransmitter system, noradrenergic, cholinergic, gamma-aminobutyric acid (GABA)ergicare also linked with olfactory bulbectomy.

It could be ended by saying that the OB rat is a perfect model for depression because

many changes which are been observed in depressed patients are also been exhibited similarly by OB rats.

Genetic models- *Fawn hooded*:

At sea level pulmonary hypertension is spontaneously developed in Fawn-Hooded Rat (FHR). Upon exposure to mild hypoxiaincreased severity of this disease takes place. Fawn hooded rat strain shows a blunted response and hypercortisolaemiato suppression induced by dexamethasone which is compatible with the HPA axis hyperactivity that is observed in depressive illness. On chronic treatment with the antidepressant drugsthe elevated levels of plasma cortisol are reduced.

4.6.6 STRESS MODELS:

Stress models are explained by saying that main stimulus is the stress, which increases changes in the behavior of animal.

Learned helplessness:

Learned helplessness is the behavior that takes place when a subject endures aversive stimuli or pain repeatedly from which it is unable to avoidor escape. After experiences like above, the animal often fails to accept or learn "avoid" or “escape” in new situations where such a behavior is effective. In other words, the animal learned that it is helpless. In some situations where there is a aversive stimuli, it has accepted that it has loss of control and thus gives up trying, even as changing circumstances offer a method of relief from said stimuli. Such an animal is known to have acquired learned helplessness. Learned helplessness theory is the view that related mental illness and clinical depressionwhich may result from such perceived or real absence of control on the situation outcome.

Tail suspension test:

An experimental method which is used in scientific research to measure stress in rodents is Tail suspension test (TST). When a rat is subjected to short term inescapable stress then the rat will become immobile, based on an observation. It is used likely to measure the potency of anti-depressant agents, but there is significant controversy over its usefulness and interpretation.

Forced swim test:

During Forced Swim Test, the animal is placed in aninescapable container filled with water. The animal initially tries out to escape but will exhibit immobility eventually (*i.e.*free floating without any movement except for those necessary for keeping the nose above water). In animal research,the FST is a very popular model for a number of reasons. First, it involves exposure of stress to the animals, which was shown to have a role in the tendency for major depression.

Restraint stress:

Some work has been carried out with this technique, either as a tool for the investigation of other physiological, pharmacological,restraint stress or pathologic phenomena itself working as the object of study. As noticed, major use of restraint stress has been for the stress responses induction in animals and, more specifically for the investigation

of drug effects, gastrointestinal effects, especially as they affect typical stress-related pathology,neuroendocrine, and immunological agents have been studied extensively.

4.7 ANTI-OXIDANT ACTIVITY

OXIDANTS AND FREE RADICALS:

Oxidation is a chemical reaction that can produce free radicals through oxidants, further leading to chain reactions that can damage the organism cells. Antioxidants such as ascorbic acid(vitamin C) or thiols can terminate these chain reactions.

Free radicals possess an unpaired electron in the atomic orbital, and can also be defined as any molecular species capable of existing independently .In some common properties,presence of an unpaired electron could be shared by most radicals. Many radicals are highly reactive and unstable. They can either acceptor donate an electron from other molecules, therefore working as reductants or oxidants .Superoxide anion radical, hydroxyl radical, hydrogen peroxide, hypochlorite, oxygen singlet, peroxynitriteradical, and nitric oxide radicals are the most important oxygen containing free radicals in many diseases. These

are the species which are highly reactive,present in the membranes of cells and nucleus destroying the biologically relevant molecules such as carbohydrates, lipids, proteins and DNA. Free radicals attack the most important macromolecules which lead to homeostatic disruption and cell damage. All kinds of molecules in the body are the targets of free radicals. Among them proteins, nucleic acids and lipids are the major targets (V. Lobo, etal., 2010)

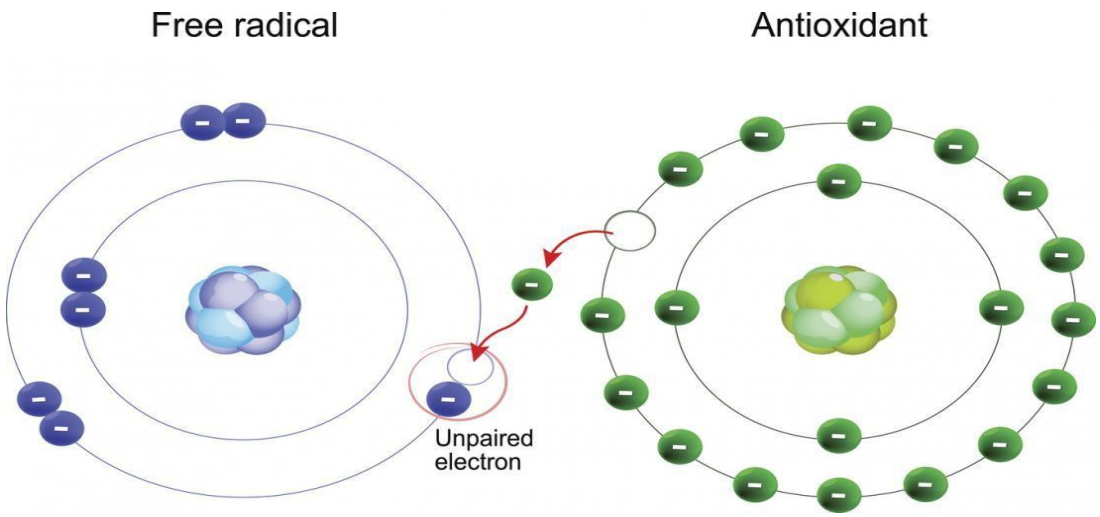

Figure 1: Role of Antioxidants and Free radicals

Production of free radicals in the human body:

Reactive oxygen species(ROS)and free radicals are derived either from external sources such as exposure to cigarette smoking, ozone, X-rays, industrial chemicals and air pollutantsor from normallyrequired metabolic processes in the human body. Formation of free radicals continuously occurs as a consequence in the cells through both enzymatic and non-enzymatic reactions. Enzymatic reactions work as source of free radicals, including those involved in the prostaglandin synthesis, cytochrome P-450, phagocytosis, and in the respiratory chain system.Free radicals can also be formed through non-enzymatic reactions of oxygen with ionizing reactions and organic compounds as well (V. Lobo, etal., 2010).

Some sources of free radicals generated internally are:

- Peroxisomes
- Xanthine oxidase
- Mitochondria

- Phagocytosis
- Arachidonate pathways
- Inflammation
- Exercise
- Ischemia/reperfusion injury

Some externally generated sources of free radicals are:

- Ozone
- Certain drugs, pesticides
- Environmental pollutants
- Cigarrete smoking
- Industrial solvents
- Radiation

Reactive oxygen species:

Some of the chemically reactive species containing oxygen are**Reactive oxygen species** (**ROS**). Examples include hydroxyl radical, alpha –oxygen, peroxides, singlet oxygen and superoxide. In a biological context, ROS plays a prominent role in homeostasis and cell signaling as they are formed as a natural byproduct from the normal metabolism of oxygen. Although, duringthe times of environmental stress (e.g., heat exposure or UV) ROS levels can dramatically increase.This results in significant damage to cell structures. Cumulatively, this is defined as oxidative stress. Plants that contain stress factor responses can influence the production of ROS; factors that enhance the production of ROS include chilling, metal toxicity, drought, UV-B radiation, salinity andnutrient deficiency. Ionizing radiation is a exogenous source from which ROS could be generated (PallaviSharma, et al., 2012).

Antioxidant protection:

Antioxidants are the compounds that inhibit oxidation. Oxidation is a chemical reaction that is responsible for the production offree radicals, further leading to chain reactionsfrom where the cells of organisms were damaged. Antioxidants such as ascorbic acid (vitamin C) or thiols could terminate these chain reactions. Animals and plants maintain complex systems of overlapping antioxidants, such as enzymes (e.g., catalase and superoxide dismutase) and glutathione, produced internally or with the dietary antioxidants like vitamin E, and vitamin C in order to balance the oxidative state (Anujyadav, et al., 2016).

The word "antioxidant" is most widely usedfor twodifferent groups of substances: naturally occurring compounds that are present in tissue food, and chemicals of industries that are added to products to prevent oxidation.

The industrial antioxidants have diverse uses:

They act as preservatives in cosmetics, food and being oxidation-inhibitors in fuels.

Dietary supplementsof antioxidants have not shown to be effective at preventing diseasesand improvement of health in humans. Supplements of vitamin E, vitamin A and beta-carotene have no positive effect on cancer risk or mortality rate. Additionally, supplements with vitamin E or seleniumalso do not reduce the risk of cardiovascular disease.

Oxidative stress:

Oxidative stress reflects an imbalance between a biological system's ability and systemic manifestation of reactive oxygen species, to repair the resulting damagereadily or to detoxify the reactive intermediates. Toxic effects can be caused by the disturbances in normal redox state of cells through the production of free radicals and peroxidesthat damages all the cell components, including DNA, lipids and proteins. Oxidative metabolismthrough oxidative stress could lead strand breaks in DNA as well as cell damage. Reactive oxygen species (ROS) is responsible for the base damage and is mostly an indirect process, e.g. $O2^{-}$ (superoxide radical), $H2O2$ (hydrogen peroxide) and OH (hydroxyl radical). However, some ROSmight act as cellular messengers in redox signaling. Thus, disruptions in normal mechanisms of cellular signaling are caused by oxidative stress (Gabriele Pizzino, et al., 2017).

Oxidative stress in humans is thought to be involved in the development of chronic fatigue syndrome,autism,Alzheimer's disease,Lafora disease,atherosclerosis, myocardial infarction, fragile X syndrome, heart failure, sickle-cell disease, infection, depression, Parkinson's disease, infection, ADHDcancer, lichen planus, and vitiligoseems to be characteristic of individuals with Asperger syndrome. However, ROS can be beneficial, as they were used by the immune system as a way to kill and attack pathogens. Through the

induction of a process named mitohormesis, short term oxidative stress is responsible for the prevention of aging.

Table 2: Various Reactive Oxygen Species and theirmatching neutralizing antioxidants

Reactive Oxygen Species	Neutralizing Antioxidants
Hydrogen Peroxide	Glutathione, Vitamin E, Flavonoids, Vitamin C, Lipoic acid, Beta carotene, CoQ10
Lipid Peroxide	Ubiquinone, Beta Carotene, Glutathione Peroxidase, Vitamin E, Flavonoids
Hydroxyl Radical	Flavonoids, Glutathione, Lipoic acid, Vitamin C, SOD

Oxidative stress and human diseases:

Imbalance between antioxidants and free radicals in the human body is termed as oxidation. Free radicals are the molecules containing oxygen which contains uneven number of electrons. As they react faster with other molecules, free radicals are also responsible for the synthesis of large chain chemical reactions in human body.

The mitochondria and Oxidative stress:

The main role of mitochondria states that in the energy metabolism, regulation of cell death has been recently emerged out as a secondary major functioning of these organelles. This in turn, is likely to be linked intimately to their role as the major intracellular source of ROS and is mainly generated at Complex I and III of the respiratory chain. Excessive production of ROS might lead to the oxidation of macromolecules and has been implicated in cell death; mtDNA mutations and ageing. ROS generated by Mitochondria also plays an important role in the release of pro-apoptic proteins and cyt c, which triggersthe apoptosis and activation of caspases. Release of cyt c takes place by two-step process which is initiated by the hemoprotein dissociation and then it binds to cardiolipin which anchors it to the inner membrane of mitochondria. Cardiolipin upon oxidation reduces the binding of cytochrome c and thus, results in an elevated "free" cytochrome c in the intermembrane space. Conversely, antioxidant enzymes of mitochondria protect against apoptosis. Mitochondrial function is been supported by a broad spectrum of antioxidant support systems and antioxidants which are nutritional modulators. Two important modulators that clinically benefit mitochondrial function are n-acetyl carnitine, which assists the transport of fatty acid transport into the

mitochondria, and N-acetyl cysteine, which stimulates glutathione synthesis of mitochodria as an antioxidant(Ott M, etal. 2007).

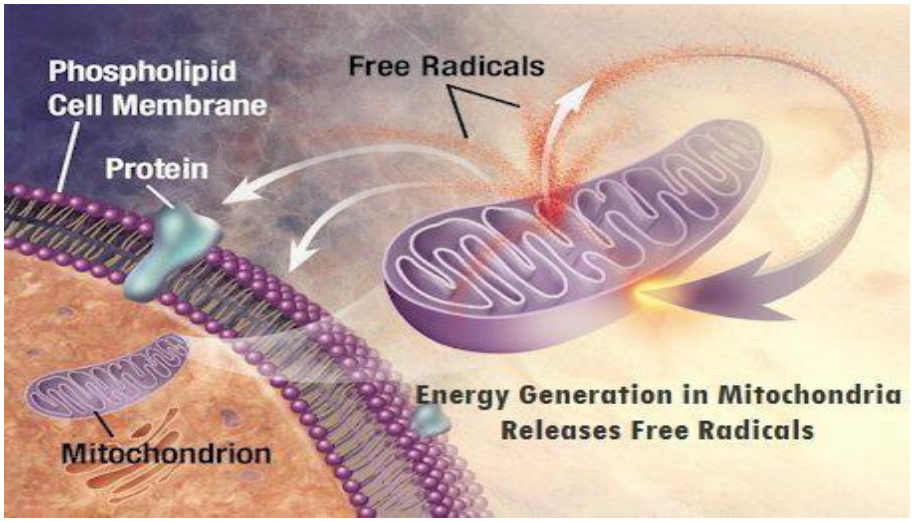

Figure 2 Generation of energy in Mitochondria

PRINCIPLES OF ANTIOXIDANT METHODS

Hydroxyl radical scavenging activity:

Hydroxyl radical scavenging activity is determined using deoxyribose assay in an aqueous medium. Reaction mixture containing EDTA (104 µM), 2-deoxy-D-ribose (2.8mM), FeCl3 (100µ M) and H2O2 (1mM) are mixed with the test extract at different concentrations. 1ml of final reaction volume was made with potassium phosphate buffer (pH 7.4, 20mM) and is incubated at 37 ^{0}C for 1 hour. The following mixture was heated in a water bath for 15 minutes at 95 ^{0}C, continued by the addition of TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA) and TCA (2.8%) 1ml each. Then the reaction mixture is ice cooled and is centrifuged for 15min at 5000rpm. The absorbance was measured at 532 nm with ascorbic acid as a positive reference [Harsha Ramakrishna, et al.,2012]

1, 1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging activity method

DPPH method was determined by taking various concentrations of test extract ranging

from 10-250µg/ml and were mixed with freshly prepared 2ml of 0.1M acetate buffer, pH 5.51ml and 1ml of 0.5mM ethanolic solution of DPPH . The tubes containing reaction mixture were incubated for 30 min at 37 ^{0}C and the absorbance was measured at 517nm with Ascorbic acid as a standard reference [Harsha Ramakrishna, et al., 2012]

DPPH radical scavenging activity was calculated using the following formula:

RSA (%)=[(Acontrol – Asample)/(Acontrol)]×100 [Harsha Ramakrishna, et al.,2012]

Hydrogen peroxide scavenging assay:

Human beings are mostly exposed to H2O2 indirectly through environment nearly about 0.28 mg/kg/day with more intake of leaf crops. Hydrogen peroxide may enter the human body via inhalation of mist or vapor and also through skin or eye contact. H2O2initiates DNA damage and lipid peroxidation in the body as it could rapidly decompose into water and oxygen, which is responsible for the production hydroxyl radicals [Frank Nagoda, 2013,].

In the process of scavenging hydrogen peroxide assay Dehpour method is used. Hydrogen peroxide solution (40Mm) was prepared in phosphate buffer at a pH of7.4, and the absorbance was measured at 560nm using a UV spectrophotometer against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging is calculated as follows:

% Scavenged (H2O2)=1-[(Ai – At)/Ai]× 100

Where Ai is the absorbance of control and At is the absorbance of test.

Reducing Power Antioxidant assay:

The reducing power assay of the test extract was determined using the method of Oyaizu. The test extract with various concentrations (200, 100, 50, 25 and 12.5 µg/mL) were mixed with 2.5 ml of phosphate buffer 6.6 containing 2.5ml of ferrocyanide are mixed with

each concentration of the test extract and were incubated for 20 minutes at 40 ^{0}C . 10% (TCA, 2.5 mL)trichloroacteic acid was added to 5ml of mixture and is centrifuged for 5min. The supernatant was mixed with 2.5mL of distilled water which contains(0.5mL) of 1% ferric chloride. Then the absorbance was measured at 700 nm. [Frank Nagoda, 2013,].

Nitric oxide scavenging activity:

Basically, nitric oxide was generated from sodium nitroprusside. Nitric oxide scavenging assay was determined by taking 5.0ml of the reaction mixture containing 5mM of sodium nitroprusside into the phosphate buffered saline solution with pH 7.3 , with or without the test extract and was incubated for 180min at 25 ^{0}C in front of a polychromatic light source (25W tungsten lamp) which is visible. The generated nitric oxide interacts with oxygen to produce nitrite ion and this will be assayed for 30 min intervalsby mixing 1.0ml of incubation mixture with same amount of Griess reagent containing 1%sulfanilamide in 0.1%naphthylene diaminedihydrochloride and 5% phosphoric acid. The absorbance of the purple azo dye (chromophore)which is formed during nitrite ion diazotization with subsequent coupling with naphthylenediaminedihydrochloride and sulphanilamide was measured at 546nm. Standard calibration curve was used to estimate the amount of nitric oxide generated with the known concentrations of sodium nitrite solutions.

Nitric oxide scavenged (%)={(A0-A1)/A0} × 100

Where A0 is the absorbance of the blank(containing all reagents except the test sample), and A1 is the absorbance of test sample[Francis M.Awah, JMPR, 2010, 4(24)].

Ferric reducing- antioxidant power (FRAP) assay:

Ferric reducing anti-oxidant power assay was based on the reduction of colorless complex (Fe3+ TPTZ complex) to blue colored complex (Fe2+tripyridyltriazine) which is formed by the donation of an electron by antioxidants at low pH. At 593nm the changes in reaction mixture was monitored. FRAP reagent was prepared by mixing 10 ml TPTZ in 40 mM HCL and 20 mM FeCl3, H2O, 300mM acetate buffer at 37 ^{0}C in the ratio of 10:1:1.

Freshly prepared FRAP was micropippeted(4ml) and is added to diluted test extract 5µl. Upon reduction of Fe3+ TPTZ (tripyridyltriazine) to Fe2+ (ferrous) an intense blue color complex was observed, thenthe absorbance was measured against a blank reagent (5 µl water with 4ml FRAP reagent) at 593nm. Thereafter, the reaction mixture is incubated at 37 ^{0}C for

30 min. All determinations should be performed thrice. Standard calibration curve was plotted using absorbance 593nm against various concentrations, with Trolox as the standard reference. The obtained values were represented as mg of Trolox equivalent /gram of sample (NilimaS.Rajurkar, 2011).

Superoxide anion scavenging activity

The reduction of nitro blue tetrazolium to a purple formazen is associated with the generation of superoxide radicals through the non-enzymatic phenazinemethosulfate-nicotinamide adenine dinucleotide system.1 ml of reaction mixture contains NBT (50 µM), phosphate buffer (20mM, pH 7.4), PMS (15µM), various concentrations of sample solution (0-20µg/ml) and NADH (73 µM). The above reaction mixtures were incubated for 5min and their absorbance was measured at 562nm against blank. Quercitin was used as a standard reference (BibhabasuHazra, 2008).

The HORAC (hydroxyl radical averting capacity) assay:

HORAC assay is based on the measurement of the metal-chelating activity of antioxidants, under Fenton-like reaction conditions. This method has its ability to protect against the hydroxyl radical formationby the formation of a Co(II) complex. Sample to be analysed is been incubated with the Fluorescein, then Fenton mixture which generates hydroxyl radical is added. The initial fluorescence was measured and readings were taken every minute after shaking. Standard calibration curve was plotted using the solutions of Gallic acid (Tingjing Zhang, 2016).

Phosphomolybdenum assay

This particular assay is having its origin on reduction of Mo (V I) to Mo (V) by the test extract followed by the subsequent formation of a Mo (V)/green phosphate complex at a pH. 1 ml of plant extracts at various dilutions (50,100,200,400µg/ml) was mixed with 1ml solution containing reagent (28Mm sodium phosphate, 4mM ammonium molybdate and 0.6M sulfuric acid). Test tubes with reaction solutions were incubated for 90 minutes at 95ºc. 30 minutes after cooling their absorbance was measured using a spectrophotometer at 695nm against blank. Ascorbic acid was used as a positive control [Narayanan ravishakaretal;,2014].

REVIEW OF LITERATURE

4. REVIEW OF LITERATURE

1. **Evaluation of antioxidant and free radical scavenging activity of Annona muricata (Santhosh Kumar Muthu, et al., 2015)**

ABSTRACT

The use of plant based remedies and their derived substances has been integral part of traditional medicine throughout the world with the discovery of new therapeutic agents and versatile applications. Annona muricata fruits commonly known as soursop have highest economic value and popularity. In the present study, 50% ethanolic extract prepared from the fresh leaves of soursop was screened for its antioxidant and free radical scavenging potential.. Tannins (0.44+0.0013 mg/g), flavonoids (1.92+0.02 mg/g), phenolics (104.43+0.013 mg/g), carotenoids (0.302+0.001 mg/g), α -tocopherol (14.80+0.02 mg/g), reduced glutathione (7.4+0.01 mg/g), lycopene (0.34+0.01 mg/g) and Vit C (1.98+0.011 mg/g) contents were found to be present. Glutathione-S-transferase (0.740+0.0031 U/g), Peroxidase (6.8+0.101 U/g), Superoxide dismutase (104.70 ± 1.88 U/g), Catalase (2.57 ± 0.02 U/g), Polyphenol oxidase (0.33 ± 0.002 U/g), and Glutathione reductase (35.89+0.103 U/g) activities were found. In addition, total antioxidant capacity (TAC) was found with IC50 values of 44.2474 µ g/ml. Radical scavenging potentials against superoxide, nitric oxide, hydroxyl, and hydrogen peroxide were found to be effective with IC50 values 59.05+0.103, 70.12+0.023, 134.21+0.063 and 43.4+0.102 µ g/ml respectively. The finding of the study reveals that hydroalcoholic extract of Annona muricata leaves could be considered as nutraceutical with potent source of antioxidants and radical scavenging activity suitable for prevention of human diseases caused by oxidative stress.

2. **Antioxidant, DNA protective efficacy and HPLC analysis of *Annona muricata* (soursop) extract(V. Cijo George, et al., 2015)**

ABSTRACT:

Annona muricata is a naturally occurring edible plant with wide array of therapeutic potentials. In India, it has a long history of traditional use in treating various ailments. The present investigation was carried out to characterize the phytochemicals present in the methanolic and aqueous leaf extracts of *A. muricata*, followed by validation of its radical scavenging and DNA protection activities. The extracts were also analyzed for its total

phenolic contents and subjected to HPLC analysis to determine its active metabolites. The radical scavenging activities were premeditated by various complementary assays (DRSA, FRAP and HRSA). Further, its DNA protection efficacy against H2O2 induced toxicity was evaluated using pBR322 plasmid DNA. The results revealed that the extracts were highly rich

in various phytochemicals including luteolin, homoorientin, tangeretin, quercetin, daidzein, epicatechingallate, emodin and coumaric acid. Both the extracts showed significant ($p < 0.05$) radical scavenging activities, while methanolic extract demonstrated improved protection against H2O2-induced DNA damage when compared to aqueous extract. A strong positive correlation was observed for the estimated total phenolic contents and radical scavenging potentials of the extracts. Further HPLC analysis of the phyto-constituents of the extracts provides a sound scientific basis for compound isolation.

3. **Exploring the Leaves of *Annona muricata* L. as a Source of Potential Anti-inflammatory and Anticancer Agents (SitiMarium Abdul Wahab, et al., 2018)**

Abstract:

The use of anti-inflammatory natural products to treat inflammatory disorders for cancer prevention and therapy is an appealing area of interest in the last decades. *Annona muricata*

L. is one of the many plant extracts that have been explored owing to their anti-inflammatory and anticancer effects. Different parts of *A. muricata* especially the leaves have been used for various ethnomedicinal purposes by traditional healers to treat several diseases including cancer, inflammation, diabetes, liver diseases, and abscesses. Some of these experience-based claims on the use of the plant have been transformed into evidence-based information by scientific investigations. The leaves of the plant have been extensively investigated for its diverse pharmacological aspects and found eminent for anti-inflammatory and anticancer properties. However, most studies were not on the bioactive isolates which were responsible for the activities but were based on crude extracts of the plant. In this comprehensive review, all significant findings from previous investigations till date on the leaves of *A. muricata*, specifically on their anti-inflammatory and anticancer activities have been compiled. The toxicology of the plant which has been shown to be due to the presence of neurotoxic annaceous acetogenins and benzyltetrahydro-isoquinoline alkaloids has also been updated to provide recent information on its safety aspects. The present knowledge of the plant has been critically assessed, aimed at providing direction toward improving its prospect as a source of potential anti-inflammatory and anticancer agents. The analysis will provide a

new path for ensuring research on this plant to discover new agents to treat inflammatory diseases and cancer. Further *in vitro* and *in vivo* studies should be carried out to explore the molecular mechanisms underlying their anti-inflammatory responses in relation to anticancer activity and more detail toxicity study to ensure they are safe for human consumption. Sufficient preclinical data and safety data generated will allow clinical trials to be pursued on this plant and its bioactive compounds.

PLANT PROFILE

5. PLANT PROFILE

5.1 PLANT INTRODUCTION

5.1.1 *Annona muricata*

Kingdom :Plantae

Clade :Angiosperms

Clade :Magnoliids

Order :Magnoliates

Family :Annonaceae

Genus :Annona

Species : A.muricata

Synonyms : Lakshmanaphalam, Graviola, Soursop, Guanabana

Common Name : Graviola

Figure 3: Shrubby Tree *Annona muricata*

DESCRIPTION

Annona muricata is aflowering, broadleafperennial evergreen shrubby tree local to Cuba, Mexico and Central Americafrom where its exact origin is unspecified. It is also native to tropical regions of Caribbean, America and is widely distributed in some parts of India. It is in the genus Annona and is in the Annonaceae family and belongs to the custard apple family but also has citrus flavor.Lakshmanaphalam and soursop are its common names while its biological name is *Annona Muricata*. It is an evergreen tree which grows upto 5-10 meters in heightand is a shrubby plantlocated mostly in the rainy forest areas but now they are been available and used locally for its various ethanomedical benefits. This is used as a purgative, laxative and also wound healing and many more.

Annona muricata tend to bloom and fruit most of the year. This grows under the conditions of 1200m below altitude and at temperatures between 25 and 28^{o}c, and at humidity nearly between 60 and 80% annual rainfall more than 1500mm. it is an edible fruit which is in dark green color (Ana V. Coria-Tellez et al, 2018).

Traditional medical uses:

The fruit, bark, leaves and seeds of *A.muricata* are mostly used as traditional medicine, mostly in south pacific countries,Caribbean islands the leaves are used in bath to treat skin ailments while in Mauritius the use of leaves are limited only to their pain site. Decoction prepared from these leaves is used as an analgesic in some countries such as Brazil, Cuba, Caribbean and Mexico. Various parts of the plant are also used to treat discomfort associated with flu, colds, asthma etc. People of Malaysia use leaves of *A.muricata*to treat and internaland cutaneous parasites. The treatment with leaves in curing malaria is also very important in tropical countries such as Togo, Cameroon,and Vietnam. Not only the fruit but its juice is also used as a treatment of hypertension, diabetes, liver,diarrhea, heart diseases and also in various types of cancers. Many people in traditional medicine use decoction of various parts of *A. muricata* for cancer and other medicaltreatments (Ana V. Coria-Tellez et al, 2018). Unripe fruits, stem bark, roots, leaves, seeds were also used as a topical insect repellants,biopesticides, bioinsecticides. The fruit of *A.muricata* is used more extensively in the preparation of candies, syrups, beverages, ice creams and also shakes.

Constituents:

Many bioactive acetogenins and essential oils are recorded to be found in *A.muricata*, those compounds are followed by the presence of phenols, alkaloids, flavonoids, tannins, saponins etc.Due to its acidic nature it is having evidences of anti-oxidant properties and hence has been evaluated in this work. Many acetogenins and essential oils are been isolated from this plant (Soheil Zorofchian et al., 2015). The properties of anti-oxidant for Annona muricata produces resistance against oxidative stress by inhibiting lipid peroxidation, scavenging free radicals and thus prevents diseases.

Medicinal uses:

On literature review it showed that the plant is investigated and various parts of the plants showed sustained activities such as anticancer, anticonvulsant, antiarthritic, antiparasitic, antimalarial, hepatoprotective, antidiabetic, antioxidant, antidepressant (Soheil Zorofchian et al., 2015) and many more activities.

Fruit and flower: used for arthritic pain, diarrhea, parasites, rheumatism, skin rashes dysentery, fever, malaria and also to elevate milk of mother after child birth.

Leaves: headaches, insomnia. Taking leaf decoction internally is used to exhibit antirheumatic and neurological effects.

Seeds: used as antihelminthic for external and internal parasites, and also to treat pains, cough and skin diseases.

Bark: antioxidant, antidepressant,antiphlogistic, antihelmenthicactivities.

Moreover hypoglycemic, anti-inflammatory, hypotensive, antispasmodic,sedative, smooth muscle relaxant effects are also attributes to barks, leaves and roots of *A.muricata*.

Side effects:

A.muricata is Possibly Unsafe when taken through mouth because it might affectnerve cells in brain and in some other parts of the body.

Pregnancy or breast feeding: Unsafe taking trough mouth during Pregnancy or Brest feeding. Parkinson's disease: *A. muricata* can make symptoms of Parkinson's disease worse.

Low blood pressure Can leads to poisoning

Nausea can kill good bacteria

Toxicity can cause miscarriage, constipation, hallucination.

DRUG INTERACTIONS:

its drug interactions are not completely discovered but some are to avoid taking graviola if you are in use with anti-hypertensives, cardiac depressants, anti-depressants or MAO inhibitors just because this fruit may increase or decrease the action of these drugs.

MATERIALS AND METHODS

6. MATERIALS AND METHODS

6.1 Plant material collection:

Annona muricata's stem bark was collected from Dwarapudi village, East Godavari district, Andhra Pradesh, India. Plant extract preparation:

Bark of *Annona muricata* has been freshly collected and cleaned free from dirt and dead materials. These barks were dry shaded and manually crushed into course powder then packed into an air tight container until it is used. The powder is subjected to maceration with ethanol for a period of one week and then subjected for hot percolation upto 8 hours. Then, the bark extract is stored in container and placed ina desiccator for 3-4 weeks to dry and then used for our research work (Khandelwal KR.Prac pharm. 2008).

6.2 Drugs and Chemicals:

Fluoxetine hydrochloride was a product of Dr.Reddy's limited, India and Imipramine was a product of Ranbaxy limited, India which has been purchased from local market Kakinada, India. Sulphuric acid, sodium phosphate and molybdate, folin-ciocalteu, aluminum chloride, hydrochloric acid used were of analytical grade.

6.3 Handling and treatment of animals:

Albino mice of either strains weighing 25 grams were taken and placed in the laboratory. There were allowed to get acclimatized to the area at room temperature and explore surroundings for getting habituated before 18 days of the experiment. The animals are bought from animal room to the laboratory. They are allowed to feed with water adlibitum and chow diet. The drug and extract administration to the animal is between 9:30-11 a.m. Micewere divided into 4 groups with six mice each.

Group -1 is positive control and receives vehicle(2ml).

Group-2 is given standard drug alone[imipramine,fluoxetine(10 mg/kg)].

Group-3 receives low dose of test extract of *Annona Muricata* bark alone [75mg/kg].

Group-4 receives high dose of test extract [150mg/kg] alone.The test and drug extracts were given to every mice and their neurobehavioral activity has been evaluated using digital photoactometer, forced swim test, open field test and tail suspension test one hour with respect to the last treatment.

6.4 PhytochemicalScreening:

Qualitative screening

Test for Saponins:

The below method is determined using the method of Chukwuma et al. 5 ml of test extract was boiled in water for 10 minutes using a water bath and then filtered. 5ml water is then added to the filtrate and vigorously shaken inorder to form a stable froth. Formed froth is been added with 3 drops of olive oil and shake well. Presence of saponin is confirmed when the emulsion is formed upon shaking.

Test for Tannins:

Test for tannins was determined by using the method of (Chukwuma et al. 0.35 grams of test extract diluted in water (1ml) was boiled in a water bath with water length upto 20 ml and filtered. Then, few drops of ferric chloride (0.1%) was added to the filtrate and left aside for the color development. Blue-black coloration or green color development results in the presence of tannins in the test extract.

Test for Phenols:

Screening of phenols is determined using the method of (Mercy Gospel Ajuru et al), for 2ml of the test extract few drops of ferric chloride solution was added and is put aside for the development of bluish green color which indicates the presence of phenols in the test extract.

Test for Flavonoids:

Test for Flavonoids was determined by using the method of Chukwuma et al. 0.30 grams of each extract was diluted with 1 ml of water and then boiled using water for 2 hours and then filtered. To the filtered test extract ammonium chloride solution (1%) was added. Upon adding the ammonium solution yellow color appears resulting in the presence of flavonoids. The diluted ammonia solution is added with H2SO4 (concentrated) and the above mixture, and put aside for few minutes, upon standing the yellow color disappears. The presence of flavonoids is indicated by the disappearance of yellow color.

Test for Alkaloids:

Presence of alkaloids is determined using the method of (Chukwuma et al). In the process of extracting the compound from the test extract, 50% ethanol is boiled along with 5% H2SO4 and then filtered using a filter paper. 28 % ammonia solution was added to the filtrate to make it alkaline. Chloroform which is diluted with H2SO4 is added to the above filtrate. The final filtrate which is extracted is the subjected for following test. Bismuth potassium iodide solution also known as Dragendroff's reagent is added to small amount of the acid filtrate and upon standing for some time the inference of orange color confirms the presence of alkaloids in test extract.

Test for Glycosides:

Screening of glycosides is determined by using the method of (Mercy Gospel Ajuru et al.,). Little amount of anthrone was added to 3ml of test extract. To make the mixture thick one drop of sulphuric acid (concentrated) was added, and is boiled on a water bath for the development of dark green color. The appearance of dark green color indicates the presence of glycosides.

Test for Steroids:

Test for steroids is determined by using the method of (Chukwuma et al).0.30 grams of test extract was mixed with ethanol (20cm3), then extracted for 2 hours. To the above extract tetraoxosulphate (VI) acid or H2 SO4and 2 ml of acetic anhydride. Upon addition green color or violet to blue color change results in the presence of steroids in the test extract. Test for Terpenoids:

Test for terpenoidswas determined by using the method of (Chukwuma et al).0.30 grams of the test extract was diluted with distilled water and chloroform (2ml) followed by the addition of H2SO4(concentrated) to the test extract (5ml)to form a layer. The formed reddish brown coloration of the interface confirms the presence of terpenoids.

Test for Phlobatannins:

According to the method of Chukwuma et al, 0.30 grams of the test extract was diluted with distilled water. The above mixture is then extracted for 24hrs and then filtered; the above filtrate is then boiled by adding aqueous hydrochloric acid (1%). Upon boiling deposition of red precipitate indicates the presence of phlobatannins.

Test for Carbohydrates:

• Molisch's Test:

To 2-3 drops of α-naphthol solution in a test tube 2ml of test extract and carbohydrate solution was added. Then using a dropper carefully pourconcentratedsulphuric acid through the walls of inclined test tube. Appearance of violet color at the junction of two liquids confirms the presence of carbohydrates.

• Fehling's Test:

To 2 ml of the test carbohydrate solution in a test tubeadd equal amounts of Fehling A & Fehling B and is boiled on a water bath for few minutes. When contents in the test tube comes to boil, then they are mixed together and formation of precipitate or color changes are observed. Appearance of brownish-red precipitate or yellow color of cuprous oxide confirms the presence carbohydrates.

• Benedict's Test:

To the test tube containing Benedict's reagent (2ml), 5 to 6 drops of the test carbohydrate solution was added and mixed well. Then the test tube is placedon a boiling water bath for 5 minutes and is observedfor any precipitate formation or color change. Then, Cool down the solution in test tube and observe the change in color from blue to yellow, red, green or orange based on the quantity of reducing sugars present in the test sample.

• Barfoed's Test:

To 2-3 mL of Barfoed's reagent, 2ml of test solution was added, mixed well and is boiled for 1minute using water bath and then set aside for some time. Immediately along side walls and bottom of the test tube a red precipitate of cuprous oxide is formed.SinceBarfoed's reagent is slightly acidic, The test is specific for monosaccharaides only.

• Seliwanoff's Test:

Two drops of test solution was added to 2 ml of Seliwanoff's reagent. Then the mixture is heated on a water bath. The appearance of cherry red condensation product will be indicates the presence of ketoses.

• Bial's Test:

To 2to3 mL of test solution add 5 mL of Bial's reagent and gently warm in a water bath for 2minutes. Indication of pentoses is followed by the subsequent formation of bluish green product whereas hexoses react generally form muddy brown products.

• Iodine Test:

To 2 mL of the test carbohydrate solution 2 drops of iodine solution was added, and is observed for the appearance of blue-black color which indicates the presence of polysaccharides.

• Osazone Test:

To 10 drops of glacial acetic acid add 0.5 g of phenylhydrazine hydrochloride and 0.1 gram of sodium acetate. To the above mixture add test solution(5ml) and is heatedusing a boiling water bath for half an hour. Slowly, cool the solution in test tube andby using a microscopeexamine the crystals. Appearance of needle-shaped yellow osazone crystals indicates presence ofglucose and fructose.

Test for cardiac glycosides

• Raymond's test:

Tofew ml of 50% ethanol add 0.1 ml of 1 % solution of m- dinitrobenzene in ethanol and test extract. 2-3 drops of 20% sodium hydroxide solution was added to the above solution. Appearance of violet colorindicates the presence of active methylene group.

• Legal's test:

Few ml of pyridine and 2drops of nitroprusside are added to the test extract and a drop of 20% sodium hydroxide solution is also added. Appearance of deep red color is observed.

• Killer killiani test:

In a mixture of 1 % ferric sulphate solution in (5%) glacial acetic acid, glycosides were dissolved. Upon adding one to two drops of concentrated sulphuric acid a blue color appears indicating the presence of deoxy sugar.

Quantitative screening:

Evaluation of phenolic contents:

Evaluation of phenolic contents was determined by using (Singleton VL et al.,). The method used to determine the phenolic contents of ethanolic bark extracts of Annona muricatais Folin-ciocalteu. To folin-ciocalteu reagent (30ml) add 0.5 ml of our test extract and 4ml solution of (20%v/v) sodium carbonate is also added to the extract after 5min. Tubes areset aside for 15min at 30^{o}c for the development of color and its absorbance is measured at 765nm using spectrophotometer. Phenolic content is estimated from the calibration curve using Gallic acid in methanol. Results were expressed in terms of Gallic acid equivalent mg/100mg dry weight.

Evaluation of Flavonoidscontent:

Evaluation of the total flavonoids content was determined using (Chang C-C et al.,) method. To our test extract add 1.8ml methanol, 3ml of distilled water, 0.1ml of 1M sodium acetate and 0.1ml of 10% aluminum chloride. Absorbance is recorded at 415nm after 30minutes. Total presence of flavonoid was determined using calibration curve with standard

as quercitin in methanol. Results are expressed byquercitin equivalent mg/100mg dry weight of our extract.

Evaluation of alkaloid's content:

Evaluation of alkaloid's content was determined using (Fazel S et al.,) method. To determine the total alkaloid presence, ethanolic bark of Annona muricata is dissolved and filtered using 2N hydrochloric acid (1mg/ml). 0.1N NaoH is added to the filtrate.1ml of this solution and 5ml of phosphate buffer along with 5ml of bromo cresol green solution is taken into a separating funnel. The test extracts were diluted with chloroform and absorbance is recorded at 470nm. Standard atropine calibration curve is used for estimating alkaloids. Total alkaloid's content is expressed using unit's mg/100mg of extract.

6.5 EVALUATION OF INVITRO ANTIOXIDANT ACTIVITY:

PHOSHOMOLYBDENUM ANTI-OXIDANT ASSAY:

*Annona muricata*bark is evaluated for antioxidant assay using phosphomolybdenum method (Narayanan ravishankar et al.,2014). This particular assay is having its origin on reduction of Mo (V I) to Mo (V) by our extract followed by the formation of a green phosphate/Mo (V) complex at a pH. 0.3 ml of plant extracts at various dilutions (0.05, 0.1, 0.3 and 0.5µg/ml) are mixed with 3ml reagent solution containing (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). Test tubes with our reaction solutions were incubated for 90 minutes at 95ºc. 30 minutes after cooling their absorbance was measured a spectrophotometer at 695 nm against blank. Mean values of our three independent samples are calculated using ascorbic acid as a positive standard reference.

Figure 4: UV- Visible Single Beam Spectrophotometer.

6.6 EVALUATION OF INVIVO ANTIDEPRESSANT ACTIVITY

Forced swim test:

Micewere individually placed and forced to swim in a transparent cylinder glass vessel(50cm height and 21 cm diameter) containing fresh water to a depth of 25cm at a room temperature of (27±2^{o}c). Overall period of immobility has been measured in a 5 minute test carried after 24 hours of 8 minutes pre-test on same mice. Mice were recognized as immobile when they stop their struggle for coming out of the cylinder and remain constant without any movement except keeping their heads above the water. Fresh water is refilled in the cylinder after every individual mice test in order to avoid bias.

Figure 5: Forced swim test

Tail suspension test:

Micewere individually placed at a height of 75cm from ground and are suspended by its tail using a plaster attached to the tail and the height. Here the mice tries out various attempts to get rid of the situation continuously and stops at a time, that is the time mice becomes motion less.That particular time is considered as immobility time and its duration is recorded in a 6 min test.

The test drug is given at 9:30 a.m. and the immobility time has been recorded.The duration of immobility in a 6 min test is recorded on a video, which is connected to a computer to avoid bias.

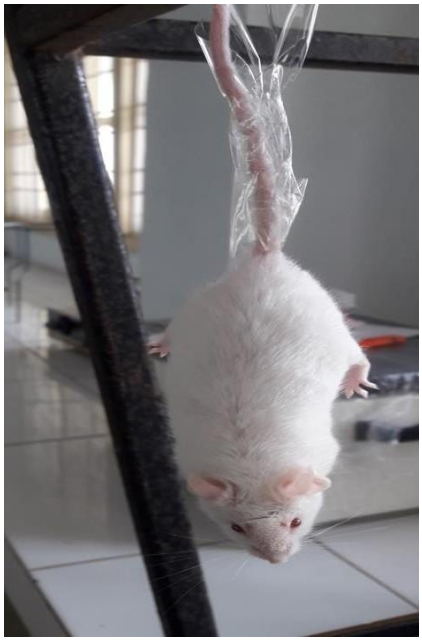

Figure 6: Tail suspension test

Digital photoactometer:

Mice were carried from animal room to the laboratory and each mice is individually placed in photoactometer and is allowed to explore the surface for 7min before test. The animal is placed in the photoactometer which is provided with a photocell light source along with digital counter. Initial score of each animal was recorded. Test drug is given to every mice alone in one group and with imipramine alone in another group. Then the activity is recorded after 30min and 1 hr. Increased locomotor activity score of mice is considered as the anti-depressant property of our test drug.

Figure 7: Digital photoactometer test

Open field test:

Animals were carried from their cages to the test room and placed individually into the open field apparatus. The Open field apparatus is constructed using a plywood and measure 50×50×40cm, which the animal could be visible. It is constructed with 16 (18×18cm) peripheral squares and 9 central squares (18×18cm). The animals are allowed to explore all around the apparatus 10 minutes prior to the test. Each animal was placed individually in the corner of apparatus, number of its crossings in center squares with all four paws, no.ofrearings, are recorded after 1 hour of test drug, vehicle and standard drug administration in all the four groups .During 5min session of observation, the number of center square entries and its frequencies are recorded and number of squares entered with all four paws, rearings were recorded. The apparatus should be cleaned with 10% ethanol solution after every individual test with mice to avoid bias for the next mice.

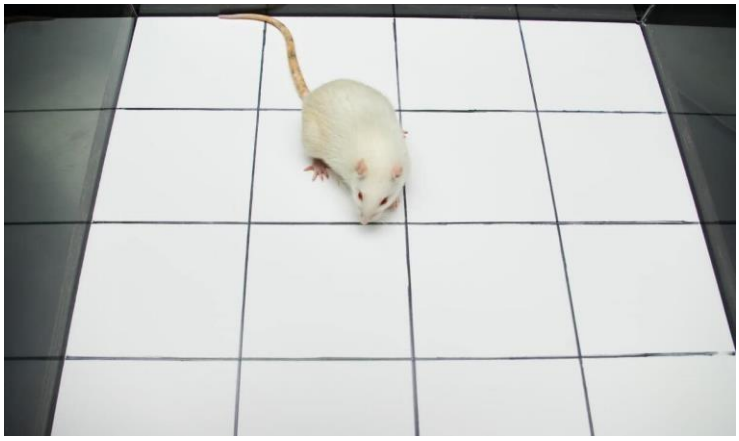

Figure 8: Open field test

Statistical analysis:

Data were analyzed by Graphpad INSTAT® version 3.0software and presented as mean±S.E.M. values. The statistical tests used were one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test. The levels of statistical significance ranged from $p<0.05$ to $p<0.001$.

RESULTS

7. RESULTS

7.1 RESULTS OF PHYTOCHEMICAL SCREENING:

The present phytochemical screening of ethanolic bark extract of *Annona muricata* showed positive results for Flavonoids, saponins, carbohydrates and phenols.

Table no 3: phytochemical screening of ethanolic bark extracts of *Annona muricata*

(Qualitative analysis)

Phytochemicals	Chemical tests	Alcoholic extracts
Alkaloids	Hager's test Dragendroff's test Mayer's test	+ve +ve +ve
Flavonoids	Lead acetate test Zinc hydrochloride test NaoH test	+ve +ve +ve
Saponins	Froth formation test	+ve
Phenols	Ferric chloride test	+ve
Carbohydrates	Molisch's test Benedict's test Fehling's test	+ve +ve +ve

Table no: 4 Quantitativedetermination of Flavonoid, Phenolic and Alkaloids content of *Annona muricata* Ethanolic Stem Bark Extract

Flavonoid (mg/g)	**Phenolic (mg/g)**	**Alkaloids (mg/g)**
44.05±0.12	35.78±0.01	22.51±0.25

All the Values are expressed as Mean ± SEM, n=3

7.2 ANTIOXIDANT ACTIVITY:

7.3PHOSPHOMOLYBDENUM ANTIOXIDANT ASSAY:

Various concentrations (50,100,200,400µg/ml) of ethanolic stem bark extract of *Annona muricata*were tested for its in-vitro anti-oxidant activity using Phosphomolybdenum assay (Table 5) revealed that, the free radicals were scavenged in a dose dependent manner.

Table 5: Effect of *Annona muricata* Stem bark extract and Ascorbic acid on free radicals by Phosphomolybdenum assay

Concentration (µg/ml)	Absorbance of *A.muricata*	Absorbance of Ascorbic acid
50	0.25±0.01	0.14±0.04
100	0.53±0.01	0.23±0.01
200	0.73±0.008	0.52±0.02
400	0.85±0.01	0.87±0.02

All the values were expressed as Mean±SEM, (n=3).

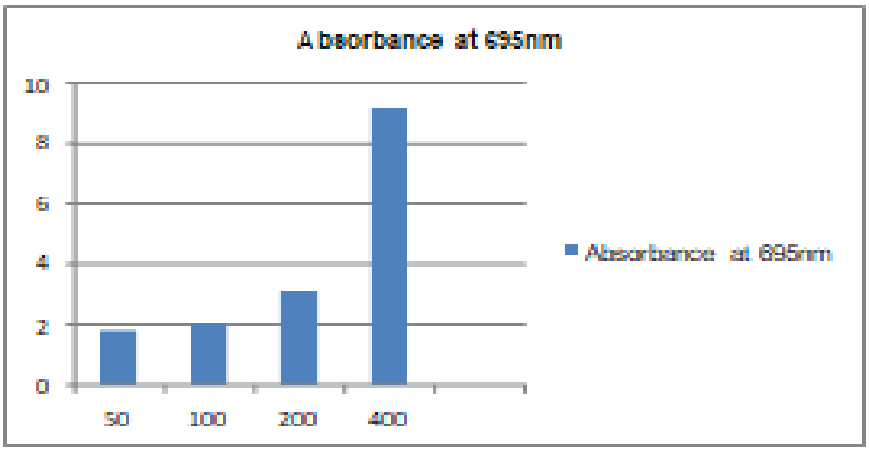

Figure9: Absorbance of ethanolic bark extract of *Annona muricata*on phosphomolybdenum antioxidant assay at 695nm.

7.4 ANTI DEPRESSANT ACTIVITY

Forced swim test:

The ethanolic stem bark extract of *Annona muricata*(75,150mg/kg p.o) when administered to mice showed significant reduction in immobility time. The results are comparable with that of standard drug Imipramine. The results were tabulated in (Table 6)

Table 6: Effect of Ethanolic stem bark extracts of *Annona muricata* using forced swim test.

Treatment	Duration of immobility Before drug administration(time in sec)	Duration of immobility After drug administration (time in sec)
Group I -Vehicle(2ml/kg)	274±1.15	273±0.57
Group II Imipramine(10mg/kg)	254±1.15	41.66**±0.87
Group III- **Ethanolic Stem Bark extract of** *A.muricata*(75mg/kg)	250±0.87	111.3*±0.87
Group IV- **Ethanolic Stem Bark extract of** *A.muricata*(150mg/kg)	272.3±0.57	57**±0.57

All the values expressed are as perMean±SEM, (n=6), * $p< 0.05$ and ** $p< 0.01$; as compared to the control.

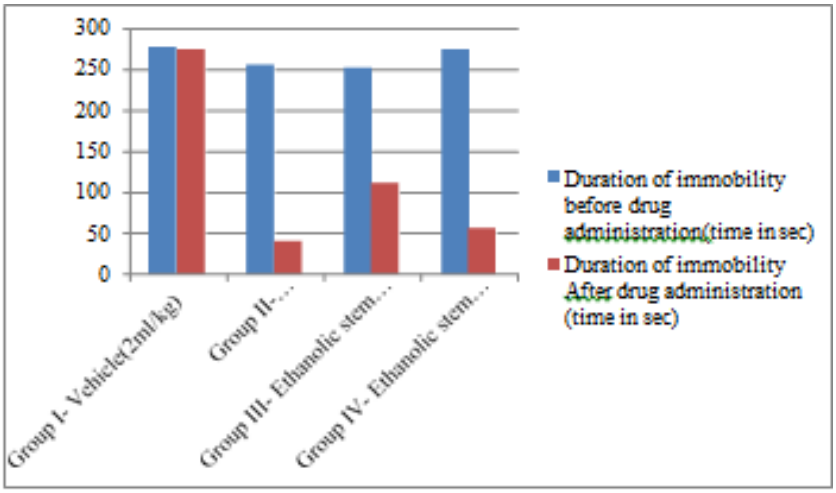

Figure 10: Effect of ethanolic bark extracts of *Annona muricata* in mice on forced swim test

Tail suspension method:

On administration of the test extract (75 & 150 mg/kg) to the mice exhibited reduced immobility time when compared to that of vehicle group. The results are comparable with that of standard drug Imipramine. The results were tabulated in (Table 5.5).

Table 7: Effect of Ethanolic Stem Bark extract of *Annona muricata*using Tail Suspension Test

Treatment	Duration of immobility in sec(before drug administration)	Duration of immobility in sec (after drug administration)
Group I –Vehicle (2ml/kg)	224.6±0.88	223±0.57
Group II Imipramine(10mg/kg)	216.6±0.88	39.6**±0.87
Group III- **Ethanolic Stem Bark extract of** *A.muricata*(75mg/kg)	224±1.15	146.6*±0.87
Group IV- **Ethanolic Stem Bark extract of** *A.muricata*(150mg/kg)	232.6±1.76	78**±0.57

All the values were expressed as Mean±SEM,(n=6), * p< 0.05 and **p< 0.01; as compared to the control

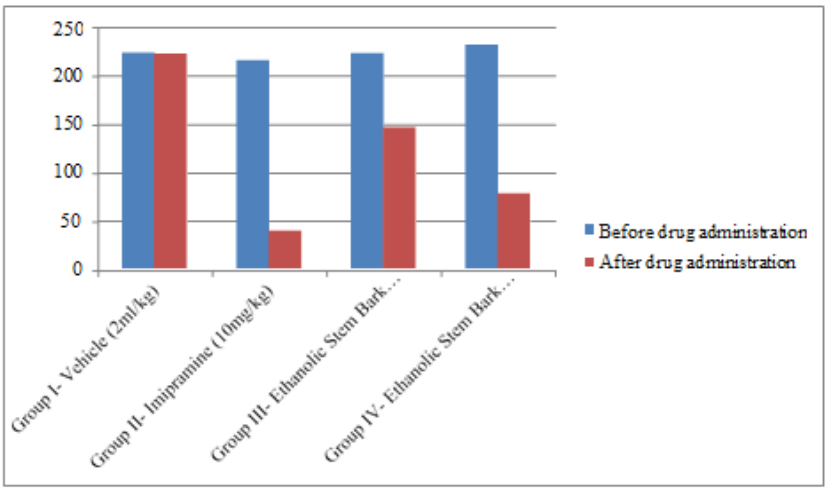

Figure 11: Effect of ethanolic bark extracts of *Annona muricata* in mice on tail suspension test

Locomotor Activity by Digital Photoactometer:

Locomotor activity of mice using digital photoactometer revealed significant increase in locomotor activity with high dose of test extract (150mg/kg) and standard drug Fluoxetine. The results were tabulated in (Table 8).

Treatment	Mean activity score before drug treatment	Mean activity score after drug treatment
Group I -Vehicle(2ml/kg)	160±1.15	164±1.15
Group II Fluoxetine(10mg/kg)	162.3±0.88	380**±0.57
Group III- **Ethanolic Stem Bark extract of** *A.muricata*(75mg/kg)	165±0.57	178*±57
Group IV- **Ethanolic Stem Bark extract of** *A.muricata*(150mg/kg)	167±0.57	288**±1.15

Table 8: Effect of Ethanolic Stem Bark extract of *Annona muricta*onLocomotorActivityusing Digital Photoactometer test

All the values were expressed as Mean±SEM, (n=6), * $p<$ 0.05 and ** $p<$ 0.01; as compared to the control.

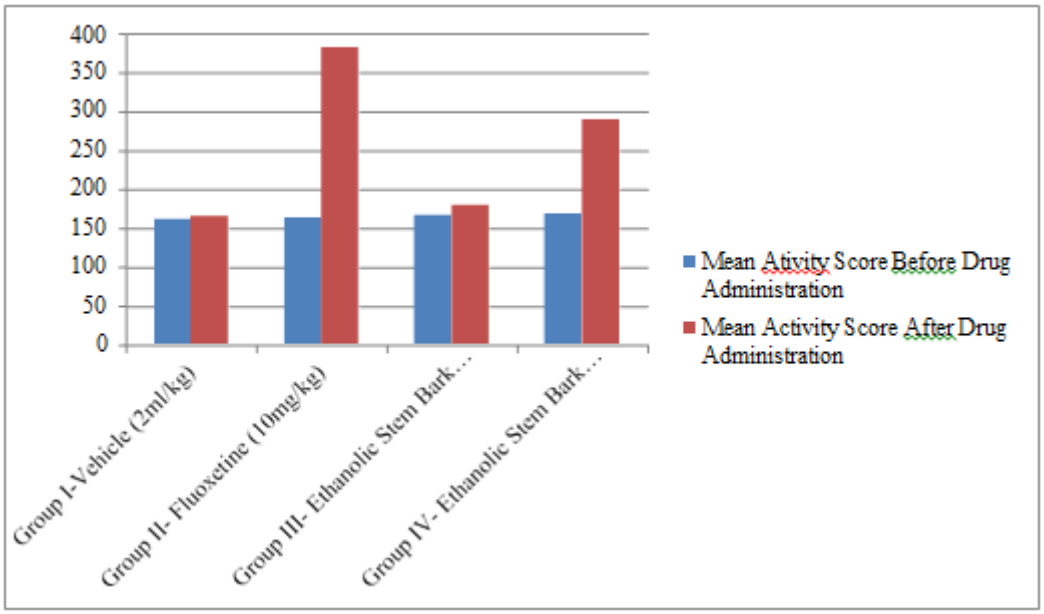

Figure 12: Effect of *Annona muricata* on locomotor activity of mice in digital photoactometer test

Open field Test Method:

Mice when placed in open field apparatus before drug administration showed more exploratory behavior.After the test and standard drug administration, the animal exhibited gradual decrease in its exploratory behavior compared to vehicle.There is significant reduction in anxiety and rearingsexhibited by mice. The results were tabulated in (Table 8 and 9).

Table 9: Effect of Ethanolic Stem Bark extract of *Annona muricta*onNo. of crossingsUsingOpen Field Test.

Treatment	No.of crossings Before treatment	No. of crossings after treatment
Group I -Vehicle(2ml/kg)	58.6±0.877	53.6±1.76
Group II Fluoxetine(10mg/kg)	54.6±0.88	40.8**±0.57
Group III- **Ethanolic Stem Bark extract of** *A.muricata*(75mg/kg)	60±1.154	42.6*±1.76
Group IV- **Ethanolic Stem Bark extract of** *A.muricata*(150mg/kg)	62.6±1.763	23.3**±1.763

All the values were expressed as Mean±SEM, (n=6), * $p< 0.05$ and ** $p< 0.01$; as compared to the control

Table 10: Effect of Ethanolic Stem Bark extract of *Annona muricta* on No. of rearings Using Open Field Test.

Treatment	No.ofrearings before treatment	No.ofrearings after treatment
Group I -Vehicle(2ml/kg)	18.6±0.87	11±0.57
Group II Fluoxetine(10mg/kg)	18.6±0.87	6**±1.15
Group III- **Ethanolic Stem Barkextract of** *A.muricata*(75mg/kg)	24.6±1.76	10.6*±1.76
Group IV- **Ethanolic Stem Barkextract of** *A.muricata*(150mg/kg)	19.6±0.88	8.3**±0.88

All the values were expressed as Mean±SEM,(n=4), * p< 0.05 and **p< 0.01; as compared to the control

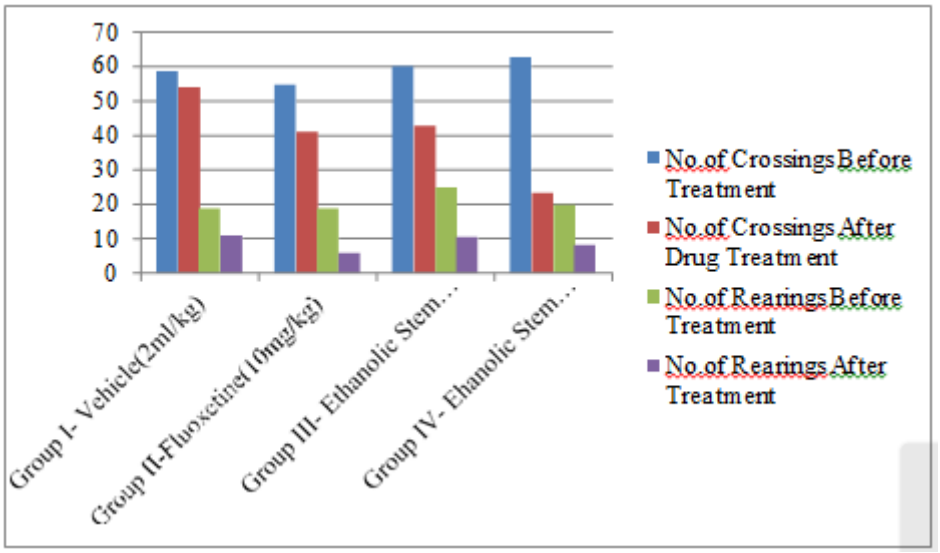

Figure 13 and 14: Effect of ethanolic bark extracts of *Annona muricata* on mice in open field apparatus test.

DISCUSSION

8. DISCUSSION

The results obtained from this study clearly indicate that the ethanolic bark extract of *Annona muricata*possess antioxidant as well as antidepressant activities.

8.1 PHYTOCHEMICAL SCREENING:

The ethanolic bark extracts of *Annona muricata*possess phytochemical constituents like Carbohydrates, Saponins, Phenolsand Alkaloids

8.2 ANTIOXIDANT ACTIVITY

Anti-oxidant activity is performed using the method of phosphomolybdenum assay, our test extract showed dose dependent manner in the free radical scavenging activity. This may be due to the presence of antioxidant property in the ethanolic bark extract of *Annona muricata*. Phosphomolybdenum assay is associated with the mechanism in reduction of phospho-MO(VI) to MO(V) and development of green color phosphate at acid pH. This assay is mostly used to elevate the anti-oxidant property of our plant extract. This is a quantitative procedure used to explore the reduction among oxidant, anti-oxidant and molybdenum ligand. At higher temperature it is involved in generating anti-oxidation thermally which gives exact estimation of reducing capability of anti-oxidant. Ethanolic bark extracts of *Annonamuricata*produced strong anti-oxidant property.

8.3 ANTIDEPRESSANT ACTIVITY

Presence of depression in the society is very high and is associated with a lot of morbidity. Hence it is very important to notify these issues for effective remedies. Since many drugs are available for the treatment, all are including some problems where there is an immediate need for an alternative for the disorders especially related with depression. Medical therapies along with herbs may be more effective for the treatment of depression. In this process,the benefits of *Annona muricata* have been studied and attempted a research work in evaluation of its antidepressant and antioxidant properties.Since many researches have gone through the evaluation of above properties, no evidences have been observed on the work of anti-depressant and anti-oxidant activity on ethanolic bark extracts of *Annona*

muricata according to literature review. So our work has taken a form, in this research work we observed that test extract doses of (75mg/kg and 150mg/kg)alone have showed very significant reduction in the immobility time of mice is found when compared to vehicle, and even more good progress is observed in immobility time in mice groups treated with standard drug(10mg/kg). Decreased immobility and increase in swimming time is found after test drug administration. This might be due to the effect of our drug is having the property to inhibit serotonin reuptake. Since stimulant drugs can reduce the immobility time in TST and FST. Immobility found in animals on FST and TST states a position of hopelessness because of its inescapable area. Various types of anti-depressants could be used to reduce immobility. The extract was found to be safe and increased locomotor activity in mice indicates the stimulant effect of *Annona muricata* showed when subjected to actophotometer. Reduced explorative activity has been observed in open field test. Reduction in its explorative tendencies after drug administration of *Annona muricata* bark extract might be due to sedative effect of our extract. Hence, its property of sedation is confirmed. Open field test yields a way to evaluate exploration, locomotion and for screening of anxiety related behavior in mice. Animals when placed in the novel environment from their acclimatized cages, after drug administration showed decreased anxiety along with stress and rearing activities. Theprogress of swimming and locomotor activities is increased only when the drug is given which increases serotonin, norepinephrine and dopamine in the nerve terminals of the animals. Decrease in monoamine oxidase activity in brain might be due to increase in all these three constituents, where as it is also proposed that depression could be of various reasons while depression might inhibit neurogenesis in the hippocampus. Overall methods were used to indicate easy use, cheap, robustness, reliability and to get accurate results. Serotonin reuptake inhibitors reduce immobility by increased swimming rather than climbing while anti-depressant drugs inhibit uptake of nor-epinephrine results in reducing immobility. The process of this research work has been supported by finding that anti-depressants can promote neurogenesis. This work would be a baseline for other researchers who have previously developed various works for future investigation of drugs for various diseases.

CONCLUSION

9. CONCLUSION

Conclusion:

It is stated that *Annona muricata* bark extracts possess anti-oxidant property along with anti-depressant property based on the results obtained. Presence of flavonoid, phenolic along with alkaloid content are present in ethanolic bark extracts of *Annona muricata* which might be used for their biological use.

The results indicate that, decrease in the duration of immobility in forced swim test and tail suspension test whencompared with the vehicle group indicates its antidepressant activity. The response from locomotor test in photoactometer confirms the antidepressant activity and differentiates from psychostimulants and tranquilizers. Results from open field test finally confirm the antidepressant activity of our extract.

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10. BIBILOGRAPGHY

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