# Pharmacological evaluation *Citrus hystrix DC* leaves ethanolic extracts rich in flavonoid content for its anti depressant activity in rat

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## 1.Introduction

According to WHO, depression is the leading cause of ill health and disability worldwide. There are more than 300 million people are now living with depression. Depression is a common mental disorder. Depression is not only about sadness, it cannot recover overnight. Depression requires long-term treatment. It is important to know that not all depression is abnormal, if you have persistent thought or suicide, you should get help right away. [1]

### 1.1Diagnosis

Diagnosis of depression can be start by consulting with a doctor or health specialist. It is essential to get the help of a health professional to rule out different causes of depression. This can be help to ensure accurate and differential diagnosis, and a more secure and cost effective treatment.[1]

DSM-V Criteria for Diagnosis of Depression

Requires 5 or more (of 9) symptoms present for at least 2 weeks.

Symptoms must include: Depressed mood and/or Anhedonia

The symptoms include:

1. Depressed mood by self-report or observations byothers

2. Loss of interest or pleasure

3. Feelings of worthlessness/guilt

4. Recurrent thoughts of death, suicide thoughts, or actual suicide attempts

5. Diminished ability to think, concentration, or indecisiveness

6. Psychomotor agitation or retardation

7. Insomnia or hypersomnia

8. Significant appetite and/or weight loss

9. Loss of energy or fatigue [1]

### 1.2 Type of Depression

The classification of depression is according to the Diagnostic and Statistical Manual of Mental Disorders, 5th ed. (DSM-V)categorical division of depressive disorders. According to the spectrum view of mood disorder, as in DSM-V, depression is not divided into independent categories. Instead, there is several type of depression lied in a continuum. There are not sharp boundaries between each type of depression. Nevertheless, a dimensional approach can be used though grading of its severity and associated features. The mood spectrum classified specific subtype of depression is useful for clinical practice. In DSM-5, the bipolar disorder is separated from depressive disorder as a separated chapter. The attention is given to duration, timing and presumed underlying causes. [3]

#### 1.2.1 Major Depression

According to the criteria published by the American Psychiatric Association, the person who is diagnosed with major depression must be present with at least 5 symptoms for at least 2 weeks. The symptoms including: sad mood, emptiness, guilt(worthlessness, hopelessness, regret) loss of energy, appetite, or interest in enjoyable activities: insomnia, tendency or acting of suicide and it is also involving changes in affect, cognition, and neurovegetative functioning. Most of the cases are highly treatable. There has never been a manic episode or a hypomanic episode. [3]

#### 1.2.2 Persistent Depressive Disorder

Persistent depressive disorder is a consistent mild or chronic depression causing clinically significant distress or impaired in functioning in an individual. These symptoms should be last for at least 2 years. The symptoms may include poor appetite or overeating, insomnia or sleeping too much, low energy or fatigue, low energy or fatigue, low self-esteem, poor concentration or difficulty making decisions, feelings of hopelessness. [3]

### 1.2.3 Premenstrual Dysphoric Disorder (PMDD)

This occurs when premenstrual dysfuntion occurs one week before the discharge of menses. The person will showing characteristic of irritability, emotional lability, headache, and anxiety or depression that remits after the menstrual cycle is over. The symptoms of PMDD are more severe than those with premenstrual syndrome (PMS). [3]

### 1.2.4 Substance/Medication-Induced Depressive Disorder

The common symptoms if this disorder including:

1.Constantly feeling sad, hopeless or empty

2. Constantly feeling irritated or agitated

3. Excessive weight gain or loss during a short period of time

4. Sleeping too much or too little

5. Low energy levels or fatigue

6. Low self-esteem

7. Poor levels of concentration

8. Decreased sex drive

9. Increased thoughts of death and dying, including suicidal thoughts and behaviour

10. The above symptoms must all have manifested during or after a specific substance/medication was taken or during withdrawal

The patient mental health history and the nature of the substance and the medication must be taken into account to confirm the type of depression during diagnosis. The substance that causing depression is There are a number of substances and medications causing this, including alcohol, phencyclidine, hallucinogens, inhalants,opioids amphetamines. [3]

### 1.2.5 Depressive Disorder Due to Another Medical Conditions

This condition is a state of depression secondary to the medical condition. Health care profession should using evidence in hand to differentiate between depressions due to medication conditions or substance induced medical conditions. Example of this medical condition is due to hypothyroidism and Cushing's syndrome.

DSM also listed some of the comorbid pathologies that are associated with depressive disorder due to another medical condition. There is evidence showing that Parkinson's disease can causing depression as one of the psychiatric condition from this disease.[2]

### 1.2.6 Other Specified Depressive Disorder

This diagnosis is includes when the condition does not meet other criteria for any specific depressive disorder. The first subtypes, recurrent depressive episode, in which that the depression last for 2 to 13 days that occur minimum once in a month. Short-duration depressive episode is occurred lasting from 4 to 14 days with non-recurrent.Depressive episode with insufficient symptoms is that when the depressive disorder last for at least 2 weeks with one of the eight other symptoms of major disorder episode. [3]

### 1.2.7 Unspecified Depressive Disorder

The diagnosis criteria according to 4 major subtypes :

a) Melancholia. A severe form of major depression having the symptoms of hopelessness, anhedonia, and psychomotor retardation, and a higher tendency of suicide. Usually occur in young adult.
b) Atypical depression. Usually occur in senior in whom there is depressed mood along with weight gain and hypersomnia.
c) Peripartum depression. The showing a symptoms of major depressive disorder in which the depression occur around parturition or within 1 month after parturition.
d) Seasonal Pattern. This depression occurs due to seasonal changes. The depressed mood occurs once in a time of year, usually winter. This may cause by the amount of sunlight that you receive affect the amount of brain chemicals or hormones that is able to affect one's mood. [3]

### 1.2.8 Disruptive Mood Dysregulation Disorder

A new diagnosis added in DSM-V and listed as a depressive disorder. This is diagnosed in children with at least 6 years old and not more than 18 years old. The symptoms are having severe temper tantrums, chronic irritability and angry mood. [3]

### 1.3 Pathophysiology of Depression

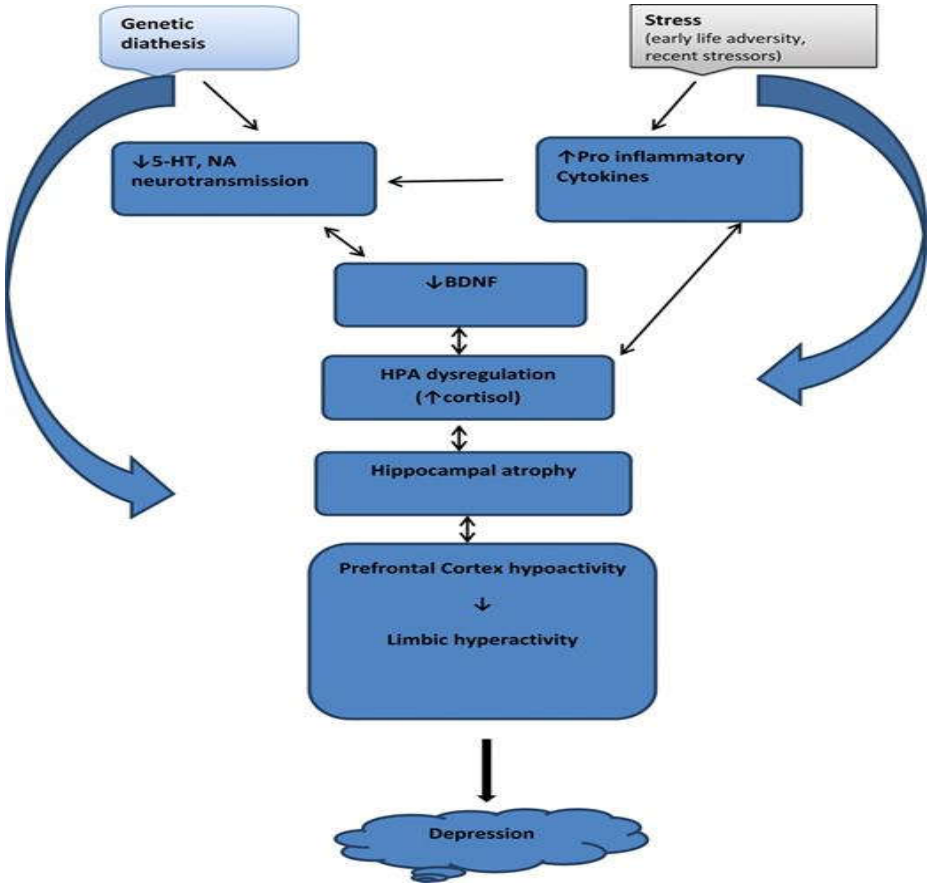



**Figure 1.3.1 : Pathophysiology of Depression**

### 1.4 Risk Factors for Depression

#### 1.4.1 Biological Factors

#### I) Genetic Risk and Familial Transmission of Depression

Evidences show that genetic factor and environmental stressor is interrelated, together with the volume of the brain, especially the hippocampus region.

The serotonin transporter gene, especially serotonin transporter promoter polymorphism (5-HTTLPR) has become a focus of the investigation. Patients with short S-allele of the serotonin polymorphism react strongly to adverse effect such as neglect, they have developed smaller hippocampal volume compares to the others. The S-allele of the polymorphism has cause decrease serotonin (5-HT) reuptake so as to decrease the amount of serotonin in the brain. Research has shown that this may due to early environmental stress and subsequent to depression. [6]

**II) Neuroendocrine Functioning**

Depression has been link to elevated level of cortisol and elevated neurohormones in the body.This is because a high level of cortisol causing a physiological change that is lead to various illnesses. For instance, the primary glucocorticoid hormone is cortisol in which it stimulates a cascade of reaction that stimulates an inhibitory response process in the HXA axis, when it fails to regulate, it causing a deleterious effect.Other than that, the activity of the gene coding for the neurokinin brain-derived neurotrophic growth factor (BNDF) is decreased after depression. Besides, adverse environmental factors, especially in the early childhood,causing the abnormal biological stress regulation. [6]

**III) Familiality**

A twin studies shows that depression is heritable from 40% to 50%. Studies have shown that if the parents have depression symptoms, the chances of the children to develop depression are higher, and the chances increase if the parents developed depressive disorder at early age. The genetic influence has a more dominant effect at a younger age such as adolescent when they encounter with environmental stressor. [3]

**IV)Alteration in Neural Structure as Risk Factor of Depression**

In the MRI scanning there is a reduced volume of hippocampus (HC), amygdala, and cingulate cortex in depressed individuals. Besides, there is various

cortical, subcortical and brain stem regions have been shown to have abnormal activation or metabolism in brain imaging studies. [5]

**V)Inflammatory System**

Recent evidence shows that chronic stress lead to inflammatory process and chronic inflammations lead to depression. The inflammatory response also contribute to symptoms of depression such as sleep dysregulation and social dysfunctioning.[6]

**VI)Neurochemicals and Transmitters Model of Depression**

Recently, the monotransmitter (norepinephrine, dopamine, serotonin and histamine)is given emphasis in mood disorder.

For dopamine monoamine, 2 recent theories have states that mesolimbic dopamine pathway may be dysfunctional in depression and that the dopamine D1 receptor is hypoactive in depression. For norepinephrine, the clinical effectiveness of the antidepressant with norepinephrine supports the role of it in the pathophysiology of depression. Besides, low level of serotonin may causing depression and some patients with suicidal tendency have low cerebrospinal fluid (CSF) concentration of serotonin metabolites as well as low concentrations of serotonin uptake sites on platelets.

Abnormal level of choline (precursor of acetylcholine) have been found in depressive patient. [6]

**VII)Sleep Dysregulation and Related Regulator of Depression**

Depression is associated with a premature loss of deep (slow-wave) sleep and increase in nocturnal arousal. Nocturnal arousal is reflected by 4 types of disturbance : (1) an increase in nocturnal awakening. (2) a reduction of total sleep time. (3) increased in phasic rapid eye movement (REM) sleep, (4) increased in core body temperature. The increase in REM drive and a decrease slow-waved sleep, the combination of both increase in REM and a decrease in slow-wave

sleep resulting in reduced REM latency. It can be used to identify 40% of depressed outpatient and 80% of depressed impatient. [4]

### 1.4.2 Cognitive

**Cognitive Theories**

Depression can be present in individual who is having specific cognitive distortion more susceptible to depression. The distortion is known as depressogenic schemata. Depressogenic schemata are cognitive templates that predicts both internal and external data that is affected by childhood experience.

According to Aaron Beck in which it postulated a cognitive triad of depression that consists of :

(1) View about self ( negative self-concept)
(2) About the environment ( tendency to experience world as hostile and demanding)
(3) About the future ( suffering and failure)

Therapy can be given to modified this distortions.

**I) Element of Cognitive Theory**

| Element | Definition |
|---|---|
| Cognitive Triad | Beliefs about oneself, the world, and the future |
| Schemas | Ways of organizing and interpreting experience |
| Cognitive distortions | An individual is convinced into something that is not true |
| Arbitrary inference | Drawing a specific conclusion without sufficient evidence |

| | |
|---|---|
| Specific abstraction | Focus on a single detail whole ignoring other, more important aspects of an experience |
| Overgeneralization | Forming conclusions based on too little and too narrow experience |
| Magnification and minimization | Over- or undervaluing the significance of a particular event |
| Personalization | Tendency to self-reference external events without basis |
| Absolutist, dichotomous thinking | Tendency to place experience into the basis of all-or-none categories |

**Table 1.4.2 : Element of cognitive theories**

**II)Learned Helplessness**

This theory related the sad event to the experience of uncontrollable events. The internal causal explanations produce a loss of self-esteem after adverse external events. To recover from it, patients should learn sense of control and mastering the environment. [3]

**1.4.3 Psychosocial Factors**

**I)Life Events and Environmental Stress**

Stress is the first episode in long-lasting changing brain biology. This is usually cause by stressful event in life. This may alter the functional states of neurotransmitter and the intraneuronal signaling system such as loss of neurons and as excessive rduction in sypnatic control.

According to DSM-5, ordinary grief and bereavement is not longerincluding into risk factors of depression. However, evidence shows that losing a parent before the age of 11 has a high probability of developing depression. Guilt may cause depression. Besides, a person who is losing a job has a 3 times more susceptibility to depression. [3]

**II)Personality Factor**

Not a specific personality trait can predispose a person to depression, however, the personality pattern of a person can made it becomes depressed under particular conditions. A person with certain personality such as OCD, botterline disorder, and historinic has a higher susceptibility to depression than person with paranoid and antisocial personality disorder. [3]

Besides, if the stressor is reacting negatively to a person self-esteem from the patient point of view, it may develop depression. What may seem to be a mild stressor to outsider may be devastating to the patient because of the event has attached to them with a different meaning. [3]

**1.4.4Psychodynamic Factors in Depression**

The theory is defined by Sigmund Freud and expanded by Karl Abraham.

Keypoints:

(1) Disturbances of mother-infant relationship during the oral phase predispose to subsequent vulnerability to depression.
(2) Depression can be linked to real or imagined object loss
(3) Defence mechanism invoked to deal with distress connected with the object loss
(4) Because the loss towards object is a mixture of love and hate, feeling of anger are directed towards themselves.

Depression will developed when the patients realized that what they expect is not going to happen. Edith Jacobson states the state of depression is similar to a helpless child has to deal with a tormenting parent. Silvano Arieti found out that many depressed people living for other rather than themselves. The person lives with a dominant others which may include principle, an ideal, an institution or an individual. [3]

### 1.5 Pathophysiology of Depression

The older hypothesis state that there is deficit in the amount of monoamine in the brain ( monoamine hypothesis), the newer hypothesis state the role of neurotropic and endocrine factor plays a major role. (neutrophic hypothesis).

Neurotrophic hypothesis nerve growth factors such as brain-derived neurotrophic factor (BDNF) are responsible for the regulation of neural plasticity, resilience, and neurogenesis. Depression is associated with the loss of neurotrophic support. By activating the tyrosine kinase receptor B in both neurons and glia, thus influence the neuronal survival and growth effect. [7]

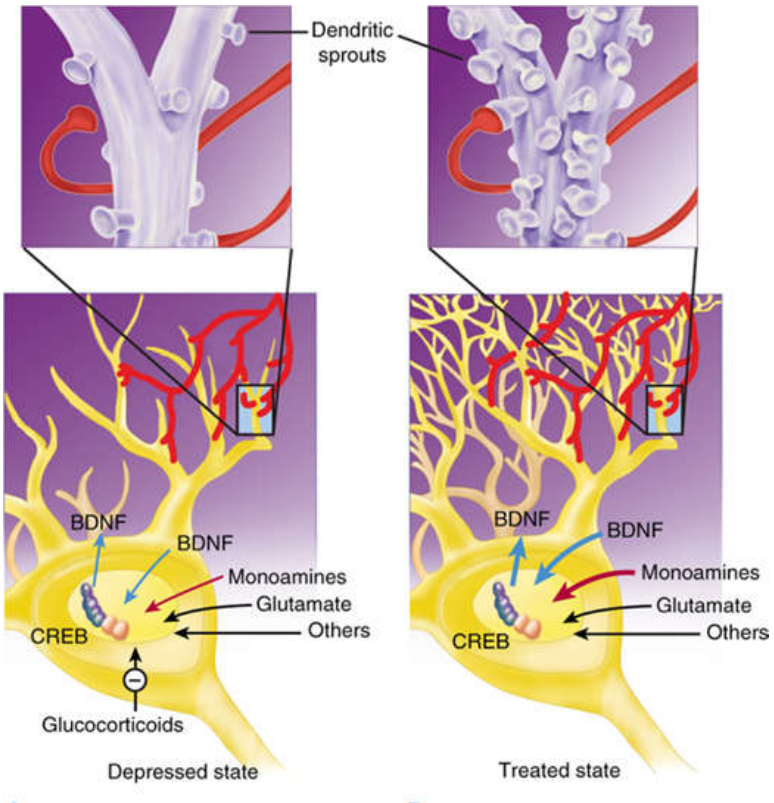


**Figure 1.5.1 : Neurotrophic hypothesis**

### 1.5.1 Monoamines and other Neurotransmitters.

Depression is link to a deficiency in the amount or function of cortical and limbic serotonin (5-HT), norepinephrine (NE), and dopamine (DA). [7]

### 1.5.2 Monoamine hypothesis

Deplete monoamine is associated with depression. Deplete catecholamine in depressed patient which is treated with noradrenergic agent before has a higher chances of relapse.

Besides, depressive patients also have changes in the function of monoamine. Studies has shown that the changes in serotonin receptor numbers (5-HT1A and 5-HT2C) or norepinephrine (α2) receptors in depressed and suicidal patients. A reduction of the serotonin metabolites in the CSF has causes violent suicide behavior.

Besides, glutamate as a neurotransmitter has its role in term of its increase in CSF has found in depressed patient. The plasma ratio of glutamate in depressed patients has decreased.[7]

### 1.5.3 Neuroendocrine Factors in the Pathophysiology of Depression

HPA abnormalities have found in depressed patient, more in patient with major depressive than in minor depressive. In terms of thyroid dysregulation, 25% of depressed patient has abnormal thyroid level in the brain. For instance, during the depressed states, the increased level of thyroxine in the circulation.

Sex steroid in women such as estrogen which are deficient in women in the postpartum and postmenopausal periods can leading to depression. Similarly, the low level of testosterone in male also precipitate depression. [7]

### 1.5.4 Integration of Hypotheses Regarding the Pathophysiology of Depression

The above hypothesis is a description and cannot mutually exclusive. Monoamine, endocrine, and neurotropic systems are interrelated in several ways. For instance, the binding of glucocorticoid receptor by cortisol leading to decrease amount of BDNF synthesis causing in decreasing in size of the hippocampus. [7]

### 1.6 Treatment for Depression

### I) Pharmacologic Treatment

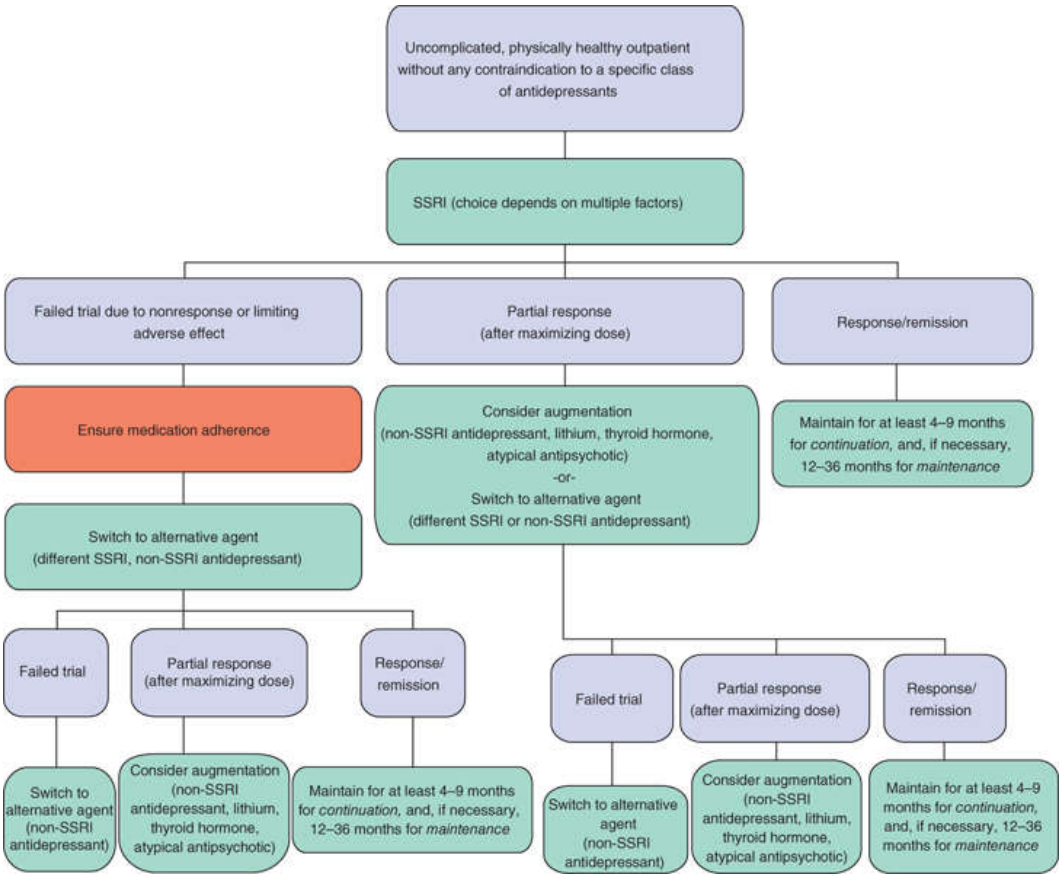



**Figure 1.6.1: Pharmacotherapy for Depression**

SSRIs: inhibits 5-HT reuptake transporter

- Fluoxetine, Sertraline, Paroxetine

SNRI: inhibits 5-HT and NE reuptake transporters

- Venlafaxine

Bupropion: inhibits DA and NE reuptake transporters

- Wellbutrin

Mirtazapine: α2-antagonist which increase 5-HT and NE

- Remeron

Triazolopyridines: increase 5-HT, NE, and DA by 5-HT reuptake transporter inhibition & 5-HT2A antagonism

- Nefazodone

TCA: inhibits 5-HT and NE reuptake transporters but not 1st line due to multiple adverse effects and possibility of fatal overdose

- Imipramine, Desipramine, Trimipramine

MAOI: inhibits breakdown of 5-HT, NE, and DA but utilized as last line due to dietary restrictions and drug interactions

- Tranylcypromine, Phenelzine, Moclobemide [7]

**II) Psychotherapy for Depression**

Psychotherapy is a talking therapy that involves a trained therapist with the patients, it can be in the form of individual, couple, and a group of people. Meta-analyses shown that psychological therapies is effective in wide range of situations. However, the size of effect is often overestimated. [8]

**Low-intensity**

The interventions is conducted by paraprofessionals based on self-help materials.Physical activity programmes is included in NICE guidelines20 under this category. [8]

**Computerised CBT (cCBT)**

A form of CBT but it is done in a computerized or online version. Between each session, there is a homework task and active behavior monitoring. A professional is there to introduce,monitor and review the outcome. [8]

**Guided self-help**

The intervention is also facilitated by a professional to introduce, monitor and review the outcome. The intervention is self-administered that is usually based on CBT or behavioral approaches. Materials that are using are specially designed books or self-help manuals. [8]

**High Intensity**

A trained therapist is there to give specific theoretical approach. [8]

**Cognitive Behavioural Therapy (CBT)**

It involves a collaboration to identify and challenge dysfunctional beliefs that is precipitating negative mood, such as to modified them and the associated behavior patterns. It involves homework and between-session activities. Group settings and a modified for relapse prevention can be delivered. [8]

**Behavioural Activation Therapy (BAT)**

This emphasizes maladaptive behaviors in relation to stress rather than cognitions. It involves activity scheduling in order to increase positive, rewarding interactions and to overcome avoidance and negative reinforcement. [8]

**Interpersonal Therapy (PT)**

A structured treatment helps patients to identify the interpersonal context that is precipitating the depression of the patient.New specific strategies is developed to address them, including encouragement, problem solving, rehearsal and review. [8]

**Behavioural Couples Therapy (BCT)**

Partners with a therapist are both involved to identify negative interactions. A more supportive ways is encourage by improving communication and changing interaction patterns.[8]

**Problem Solving Therapy (PST)**

Therapist and patient collaborate to identify key problem areas and breaking down into more manageable problems and identify coping strategies.[8]

**Brief Psychodynamic Therapy (BDT)**

Using psychodynamic approaches, the therapist and patient explore recurrent internal and relationship conflicts, this include relationship from childhood and historical patterns. The aims is to understand the problem, what they have influenced the present and retrieve the problem to bring about a changes. [8]

**Supportive Couselling (SUP)**

A nondirective therapy that is using the exchanging the emotions and experiences. The process is non-judgemental listening and empathy. The patient is not taught specific skills during the intervention.[8]

**III) Electroconvulsive Therapy (ECT)**

Electroconvulsive Therapy is done under the conditions of general anesthesia. It provides electric current to the brain to produce a brief seizure. Thus, it brings a changes in the brain chemistry to revert the condition of mental illness. The treatment is used when pharmacotherapy and psychosocial therapy is

unsuccessful. The risk of the ECT is short-term memory loss and bilateral ECT has a possibility in long-term memory loss. [9]

# 2.LITERATURE REVIEW

## 2.1*Citrus hystrixDC*Taxanomy

Taxanomically, *Citrus hystrix DC* belongs to family Rutaceae, a small tree from Papada Subgenus. [10]

Kingdom Plantae : Plants

Subkingdom Tracheobionta : Vascular plants

Superdivision Spermatophyta :Seed plants

Division Magnoliophyta :Flowering plants

Class Magnoliopsida : Dicotyledons

Subclass : Rosidae

Order :Sapindales

Family Rutaceae :Rue family

Genus Citrus L.:citrus P

Species :Citrus hystrix DC. P [10]

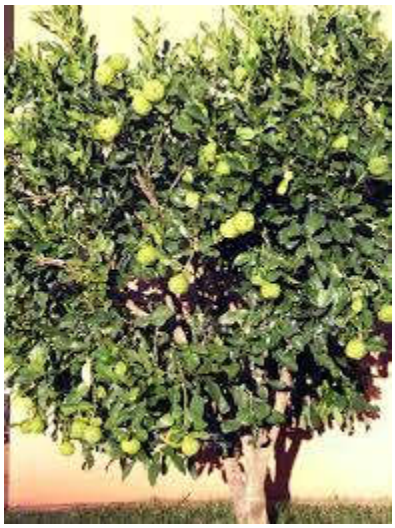


**Figure 2.1.1 : Tree of *Citrus hystrix DC***

**Figure 2.1.2 : *Fruits of Citrus hystrix DC***

**Figure 2.1.3 : Leaves of *Citrus hystrix DC***

## 2.2*Citrus hytrixDC* Morphology

The plant is native to Indonesia, Malaysia and Thailand. *Citrus hystrix DC*is a small tree that is 3 to 6m high with glabrous spiny branches.

The main focus is the leaves of the *Citrus hytrix DC*. They are having a characteristic shape of winged petioles. It is grow in an alternate form, unifoliolate, ovate shape, the length of the leaves is 7.5 to 10 cm long, light to dark green adaxial, with a smell of fragtant. The base of the leaves is rounded, apex obtuse with a smell of fragrant. Petiole is long and expanded into prominent wings,15cm long, 5cm wide leaves is a popular herbs in making Asian cuisine, primarily “Tom Yam Soup” from Thailand. [11]

The folwers are small,fragrant smell, white in colour petal of 4 to 5 pieces with ovate-oblong shape.

The fruit of the *Citrus hystrix DC* is inedible, when the fruit is ripe , the acrid oil is present in the pup cell. The fruit has almost no juice, use primarily in medicinal purpose. The size is 5 to 7cm in diameter. Seeds are numerous, ovoid-oblong in shape, 1.5 to 1.8 cm long, 1 to 1.2 cm wide, 0.5 cm thick. It is monoembryonic with white cotyledons. [11]

**2.3 Chemical Constituents**

The leaves of the plant contain alkaloids, flavonoid, saponins, tannins, terpenoids compound.

The ethanol extract (95%) of *C. hystrix* dried leaves was found to contain phenolic acids (e.g. p-coumaric acid, m-coumaric acid, benzoic acid, cinnamic acid, sinapic acid, vanillic acid).

The mixture of methanol and dimethyl sulphoxide (1:1) extract showing it contents of flavanone glycosides, eriocitrin, neoeriocitrin, narirutin, hesperidin, neohesperidin, didymin and flavone glycosides. [11]

**2.4Reports on the leaves of *Citrus hystrixDC*leaves**

The following are the report about *Citrus hystrixDC*:

**I) Medicinal uses**

Traditionally, *Citrus hystrix DC*has some uses but it is not supported by medicinal and experimental data. The *Citrus hystrix DC* leaves are used as insecticides and stomachache related to dyspepsia.

The leaves are promoting dental health by rubbing fresh leaves onto the teeth and gum. The aromatic oil has uses in perfume and medicinal uses. [12]

## II) Biological and pharmacological activities supported by experimental data

### 1)Anti-cancer effect in cervical cancer and neuroblastoma cell lines

Natural products are used as therapeutic agents against cancer has become widespread in recent years, particularly considering the toxicity of chemotherapeutics. Taken together, *Citrus hystrixDC* leaf extract reduced the viability of cervical cancer and neuroblastoma cells in micromolar concentrations. These results are in concert with aprevious study in which *Citrus hytrix DC*extract was shown to exert anti-cancer effects against human mouth epidermal carcinoma (KB) and murine leukemia (P388) cell lines,HL60 (promyelocytic leukemia), K562 (chronic myelocytic leukemia), Molt4 (lymphoblastic leukemia) and U937 (monocytic leukemia)5 cells. Further work should be undertaken to determine the anti-cancer effects of the investigated extracts in vivo and to identify the active ingredient. [12]

### 2)Cytotoxicity and apoptosis induction

Leaves contain polyphenol and essentials oil thus they are able to kill the cancer cells by performing the cytotoxicity effect. Ethanolic and ethyl acetate extract able to induce apoptoxis by increase the number of cancer cells to cervical cancer cell. [13]

### 3)Hepatoprotective

Hepatoprotective is due to the antioxidant potential. Leaves extract can reduce ROS such as to reduce the oxidative stress to the liver hepatocytes and to increase the liver antioxidant enzymes. CHEE also stimulate the regeneration through synthesis of protein and increase the detoxification of liver. [13] Treatment with the leaves extracts proves that it can stabilized the plasma membrane of liver cells and repair of hepatic tissue cause by paracetamol toxicity. [14]

**4)Antimicrobial**

*Citrus hystrix DC* leaves extract able to inhibit S. mutans activity, which may be useful in the prevention of biofilm formation on dental surface, reducing dental plaque and decreasing the chance of dental carries. [15] Formation of biofilm by S. mutans was also inhibited by the extract. These results were confirmed by the down-regulation of genes associated with the biofilm formation.

**5) Boosting the Immune system**

Methanolic extracts of leaves show 32% and 43% higher anti-inflammatory effects (by two glycerolipids : 1,2-di-O-α-linolenoyl-3-O-ß-galactopyranosyl-sn-glycerol (DLGG), 1-O- α-linllenoyl-2-O-palmitoyl-3-O- ß-galactopyranosyl- sn-glycerol (LPGG) respectively). The inflammation is in the form of edema induced by 2-O-tetradecanoyl-phorbol 13-acetate (TPA). [16]

**6) Antifungal**

Alcoholic and aqueous extract of *Citrus hystrix DC* showed significantantifungal activities against Candida albicans and Aspergillus niger.The efficacy of antifungal towards the pathogenic otomycosis was notedsolvent dependent. [17]

**7) Antitumor activity**

DLGG and LPGG (810 nmol/20 µL in 15% MeOH in CHCl3) isolated from methanol extract of C. hystrix fresh leaves ,as a result, DLGG was found to significantly ($p<0.05$) inhibited TPA-induced edema by 32%; while LPGG significantly ($p<0.01$) inhibited TPA-induced edema by 43% compared to indomethacin (19%). [11]

### 2.5 The Effect of *Citrus hystrix* Leaves Act on Depression

#### I) Anticholineterase activity

Research on AChE inhibitors activity is growing, the investigation base on AChE inhibitor has been investigated for many neurological disease. *Citrus hystrix DC*(Combava), which caused 10% inhibition of AChE enzyme and that this action was related to the presence of acyclic and monocyclic monoterpenes such as citronellal, β-phellandrene,d-limonene, present in the essential oil extracted from the leaves of this plant. This extraction able to improve memory and cognitive function in depression.

Depression can be prevented by increasing levels of acetylcholine in the brain through inhibition of the enzyme acetylcholinesterase(AChE) and butyrylcholinesterase (BuChE). These enzymes are responsible for the hydrolysis of acetylcholine to acetate and choline, which prevents its action to the neurotransmitter. Acetylcholine (Ach) is a neurotransmitter, helps in boosting memory and learning. Disturbance in Ach production and release causes fatal disease like Alzheimer and neuromuscular disorders. [18] The extraction able to antagonize the action of acetylcholinesterase by antagonizing the action of acetylcholinesterase.[19]

#### II) Antioxidant and Free Radical Scavenging Activity

High phenolic and flavonoid content and exhibited good antioxidant activity by DPPH and FRAP methods. The use of *Citrus hystrix DC* as a natural antioxidant source appears to be an alternative to synthetic antioxidants. Oxidative stress can cause depression. The antioxidant effect helps to combate with the ROS species by preventing the damage in brain cause by it. Flavanoids may act on intracellular signaling cascades(mitogen-activated protein kinase(MAPK), to prevent oxidative damage and mitochondrial function damage. This can prevent neuronal cell death. Flavanoid contents can exert effects on survival via the P13K/ Akt cascade. [20]

### III) Anti-inflammatory Activity

Rutin, a type of flavanoid protects rats from the stress-induced damage and neuroinflammation induced by streptozocin. The neuroprotective effects is available when the flavonoid is able to cross the blood-brain barrier. (BBB) Neuroinflammation is able to drive depression. [21]

Microglia mediated inflammation can depression. Epigallocatechin gallate (EGCG), a compound of polyphenol found in leaves of *Citrus hystrix DC*has also been found to attenuate infrasound-induced neuronal impairment by inhibiting microglia-mediated inflammation. [22]

### IV) Membrane Stabilizing Properties

The use of antidepressant is unrelated to the membrane-stabilizing properties. (m.s.a). However, the lipohilicity is important for the component to penetrate blood brain barrier (BBB). The greater of the membrane stabilizing activity, the more likelihood for the compounds to penetrate through central nervous system. [23]

CHEE has significantly reduce the haemolysis of RBCs in hypotonic solution (74.4%) compared to acetyl salicylic acid (93.24%) in 0.10 mg/ml. [24]

### V) Conclusion

A hypothesis is being made that combination effect of anticholineterase, antioxidant, and anti-inflammatory action from the content of the leaves of *Citrus hystrixDC*that is able to act on depression. Further research is needed to verify the activities, the coumpouds with its concentration and which activity has a more significant influence on depression.

# 3. AIM AND OBJECTIVES

Herbal medicine is also called botanical medicine or phytomedicine. It is refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes.Herbal medicines is using as a treatment in clinical in local or regional practice. Herbal medicine may replace conventional drugs if conventional drug does not work. It has a mild, gentle effect than conventional medicines and renders a lesser side effect. Today's plant-based drugs treat a range of disease, from headaches to cancer. [25]

Herbal medicine has a high demand in the market. This may cause by the reason such as higher effectiveness compare to modern medicine, cost effective, consumer belief that the natural drug is more safer, patient want to prevent the wrong diagnosis from the physician so as to use herbal medicine as an alternative and a move towards self-medication. [26]

*Citrus hystrix* is an common herbs that can be found easily in Southeast Asia. It medicinal uses cure toward all kind of disease is still undergoing research. The plant is not studied for its effect in the antidepressant thereby our research objective was basically to explore the CHEE potential for its in-vivo antidepressant activities.

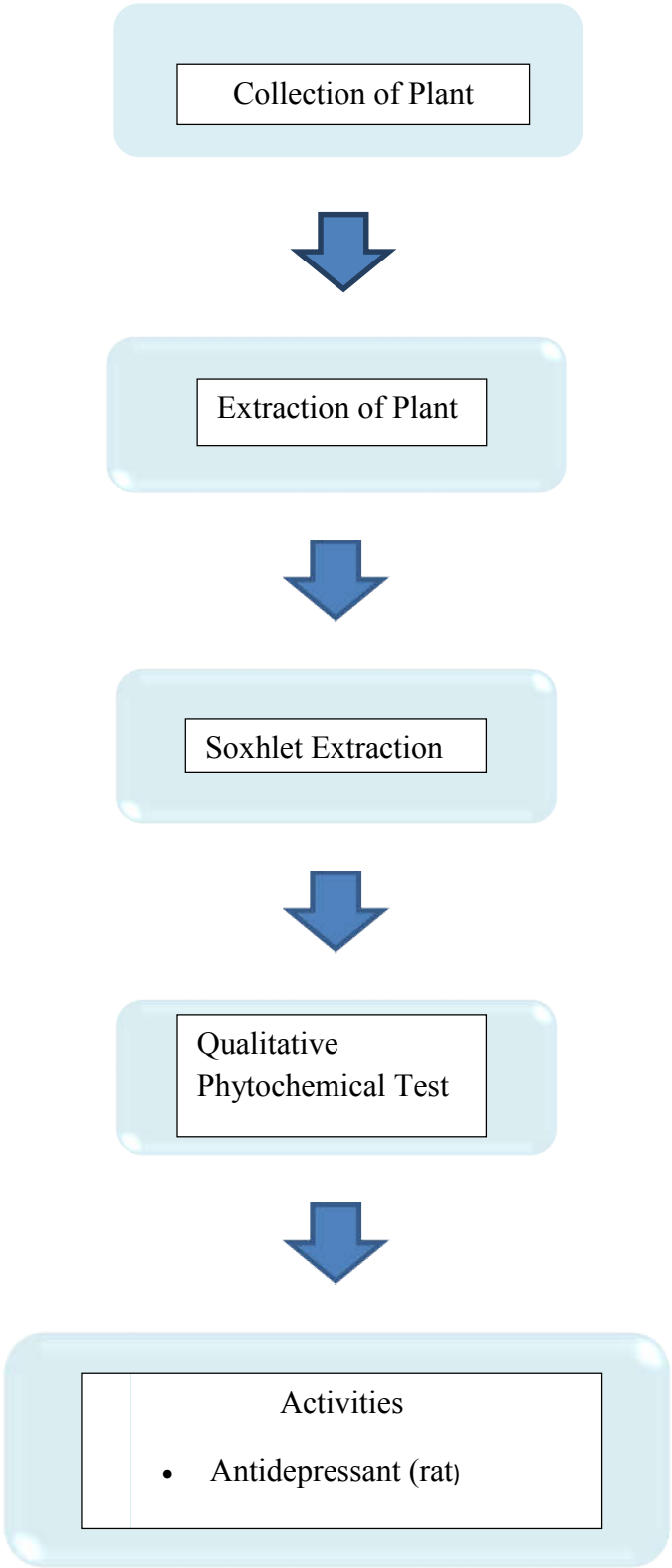
Anticholineterase and anti-inflammatory effect in the leaves of Citrus hystrix has the potential to treat depression. This is because there are some of the antidepressant works in this way. Hence, our objective is to determine the effect of CHEE in animals.

Furthermore, studies also suggest that the plants rich in flavonoid content which can helps in combating oxidative stress and neuroprotective effect in treating depression.*Citrus hystrixDC*has shown the presence of flavonoid which may contribute in antidepressant activity. Thus, research is aimed to investigate the antidepressant activity depends on the presence of flavonoids.

## AIM AND OBJECTIVES

Plant *Citrus hystrixDC*is yet to be studied for many pharmacological activities, considering these facts we aimed to perform pharmacological evaluation of *Citrus hystrixDC* leaves ethanolic extract for its antidepressant studies in rats.

## 4. PLAN OF WORK



# 5. MATERIALS

## 5.1. Antidepressant

### 5.1.1 Preparation of standard drug solution and *Citrus hystrixDC*ethanolic extract

- Citrus hystrix *DC*leaves were collected from EcoGarden Asia Plant Nursery, Sungai Petani. They were dried, blended and extracted.
- 20mg Fluoxetine is dissolved in distilled water.
- 100 mg/kg, 200 mg/kg and 400 mg/kg of *Citrus hystrixDC*ethanolic extract solution were prepared

### 5.1.2 Storage of standard drug solution and *Citrus hystrixDC* ethanolic extract solution

Fresh standard and *Citrus hystrixDC* ethanolic extract solution were prepared for each day's work. The solution were kept in screw-capped test tubes and labeled. They were kept in refrigerator until use.

### 5.1.3 Test dose and route of administration

The dose of drug for each animal was calculated based upon the body weight of the animals. All drugs were administered as per screening model procedures.

### 5.1.4 Experimental Animals

Sprague-Dawley (SD) rats were obtained from AIMST University animal house of either sex wighing 160-200gm and were housed in groups of 5. All animals were maintained at 22±1°C with 60% relative humidity, and kept under a 12-hour light dark cycle. The animals were allowed to acclimatize to laboratory conditions prior to experimentation. All experiments were conducted during the light period of 12 hours of the day/night cycle.

### 5.1.5 Ethical clearance

The experimental protocol for the entitled study was approved by AIMST Univeristy Human & Animal Ethics Committee

### 5.2 Apparatus and Equipment

- Beakers
- Volumetric flasks
- Measuring cylinder
- Weighing machine
- 5ml syringes
- Oral feeling needles
- Spatula
- Pipettes
- Blender
- Aluminium foil
- China dish
- Gloves and cloth
- Conical flasks
- Soxhlet apparatus
- Heating mantle
- UV analyser

To prevent contamination, standard working and operating procedures were strictly followed. Personal hygiene was maintained.

# 6. METHODS

## 6.1 Collection and Extraction of *Citrus hystrixDC*leaves

*Citrus hystrixDC*leaves were obtained from EcoGarden Asia Plant Nursery, Sungai Petani, Kedah, Malaysia. The leaves were collected and stored in plastic bags with enough aeration. One part of the leafy branches was kept for hebarium recording purpose.


Figure 6.1.1 : Blended powder of *Citrus hystrix DC*

## 6.2 Procedure for Extraction

Soxhlet extraction was chosen as the method of extraction in this study. The leaves of *Citrus hystrixDC*were separated from the branches and dried in a hot air oven with a temperature of 50°C. After drying, the leaves were blended and well stored for further usage.

### Soxhlet Extraction

Adequate amount of finely grounded leaves were placed in a porous bag called “thimble” which is made from cellulose. A set of Soxhlet apparatus including a round bottom flask, an extractor and a condenser was set up before starting the extraction. The thimble with leaves was placed in the thimble chamber of the Soxhlet extractor which was connected to the condenser and

round bottom flask. The round bottom flask was filed with the solvent chosen for the extraction which is absolute alcohol until half of the flask. Porcelain chips were added in the round bottom flask. The round bottom flask was filled with the solvent chosen for extraction which is absolute alcohol until half of the flask. Porcelain chips were added in the round bottom flask to prevent bumping of solvent. Two pipes were connected to the inlet and outlet of condenser. One end of the inlet pipe was connected to the hose while the other end was connected to the inlet of condenser. Another pipe was connected to the outlet and one end was left in the basin to allow exchange of fresh water during extraction. The extraction solvent was heated by a heating mantle, vaporized into the thimble, condensed in the condenser and dripped back. When the liquid content accumulated in the extractor reached the siphon arm, the liquid contents were emptied into the bottom flask and the process was cycled. The process was continued until the solvent condensed in the siphon became colourless.

**Figure 6.2.1 : Soxhlet apparatus**

### 6.3 Evaporation

After extraction was done, the ethanol solvent was evaporated using a rotary evaporator at a temperature of 60°C. When small amount of ethanolic extract was left, the remaining extract was transferred to a China dish and kept on a water bath of 55°c for complete evaporation of ethanol solvent. Finally, the extract as collected and kept in refrigerator.

**Figure 6.2.2 : Thick extracts of CHEE**

**Figure 6.2.3: Rotary evaporator**

### 6.4 Qualitative Phytochemical Test

A small quantity of extract was tested to identify the presence of various phytochemical compounds. The extracts were dissolved in 70% ethanol.

**Tests of Alkaloids:**

i) Mayer's test ( Potassium Mercuric Iodide):

1ml extract solution + few drops of Mayer's reagent give creamy white precipitate

ii) Dragendroff's test ( Solution of Potassium Bismuth Iodide):

1ml extract + few drops of Dragendorff's reagent give reddish brown precipitate.

**Test for Flavonoids:**

**i)** Lead acetate tests:

1ml of extract + few drops of 10% lead acetate give yellow precipitate.

ii) Shinoda test:

1ml extract + 0.5g magnesium fillings and 2 drops of concentrated HCL give pink or crimson red color.

iii) Alkaline reagent test:

1ml extract + few drops of 10% sodium hydroxide give intense yellow color.

iv) Ammonia test:

1ml extract + 1ml of 10% ammonia solution give yellow fluorescence.

**Test for Mucilage:**

1ml of extract was suspended in water and few drops of ruthenium red were added. Pink or red color change is observed.

**Test for Tannins:**

i) 5% ferric chloride test:

1ml extract + few drops of 5% ferric chloride solution give green color.

ii) Potassium dichromate test:

1ml of extract + few drops of potassium dichromate give red precipitate.

**Test for Gums:**

1ml extract + 5ml absolute alcohol and stirred slowly. Presence of precipitate can be observed.

**Test for Glycosides:**

Sodium hydroxide reagent test:

1ml extract dissolved in 1ml water, few drops of sodium hydroxide solution was added, gives yellow color.

**Tests for starch:**

i) Iodine test:

1ml extract + few drops of dilute iodine solution and mixed well. Blue black color change can be observed.

ii) Tannic acid test:

1ml extract + 1ml of 20% tannic acid show presence of precipitate.

**Tests for Saponins**

i) Honey comb test:

1ml extract + few drops of sodium bicarbonate solution and shake vigorously for 3 minutes. Formation of honey comb froth shows positive result.

ii) Foam test:

1ml extract + 20ml of distilled water and shake for 15 minutes. Formation of 1 cm of broth shows positive result.

**Tests for Protein:**

i) Lead sulphate test:

1ml extract + few drops of 40% sodium hydroxide, heated at direct flame for 5-10 minutes, cooled to room temperature and few drops of 10% lead acetate solution were added. Formation of black precipitate shows positive result.

ii) 5% copper sulphate test:

1ml extract + 1ml of 5% sodium hydroxide, mixed well and 1% copper sulphate solution was added. Positive result gives violet color change.

**Test for Monosaccharides:**

Barfoead's test:

1ml extract + 3-5ml Barfoead's reagent, water bath for 3 minutes.

**Test for Steroids:**

Salkowski test:

1ml extract + 1ml chloroform + 1ml concentrated sulphuric acid give brown ring.

**Tests for Phenols:**

i) Ferric chloride test:

1ml extract was dissolved in 5ml of distilled water and few drops 5% ferric chloride solution were added. Dark green color change shows positive result.

ii) Lead acetate test:

1ml extract + 0.5ml of 1% lead acetate solution show precipitation.

**Test for Amino Acids:**

i) Ninhydrin test:

1ml extract + few drops of Ninhydrin reagent, boiled at boiling water bath for 10 minutes. Positive result shows purple color change.

ii) Millon's reagent test:

1ml extract + 1ml Millon's reagent, boil in boiling water bath and cool at room temperature. Formation of red precipitate shows positive result.

**Test for Carbohydrate:**

Molisch test:

1ml extract + few drops of Molisch reagent mix gently and 2ml of concentrated HCL or sulphuric acid is added. Formation of purple or reddish violet ring in the interface of 2 solutions shows positive result.

**Tests for Reducing Sugars:**

i) Fehling's test:

5ml Fehling's solution A + 5ml Fehling's solution B, mix well and keep in boiling water for 1 minute. 1ml of Fehling mixture was mixed with 1 ml of extract and undergo water bath at 60°C. Green suspension that turns to red precipitate shows positive result.

ii)Benedict's test:

1ml extract + few drops of Benedict's reagent, water bath for 3-5 minutes. Positive result shows brick red precipitate

**6.5 Antidepressant Studies**

**i) Forced swimming test (FST)**

Animals were divided into 5 groups with 5 rats each. Group one rat was treated with distilled water (normal control). Group 2 rat were treated with standard drug Fluoxetine (20mg/kg). Group 3,4, and 5 rats were treated with 3 different doses of ChEE which were 100, 200 and 400mg/kg respectively by oral route. All rats were treated for 10 days and prior to the forced swimming test. The rats were exposed to a 15 minutes' pre-test swimming session at 24 hours prior to the end of treatment. A 6 minutes' test swimming session was carried out on the next day. Rats were individually forced to swim in a plastic cylinder of height 35

cm, diameter 25.5cm and containing water of height 25 cm with temperature maintained at 25°C. The rats were removed from water and them placed in warm enclosure before placing into respective cages. Cylinders were cleaned and filled with fresh water after performing test with each rat. In the 6 minutes' test swimming session the behavior of the rats was recorded for last 4 minutes. The behavior of each rat was recorded as videotapes and stored as video files. A time-sampling technique was used, whereby the behavior in each 5 seconds interval of the 4 minutes swimming test was scored. The behavior of the rats was judged as one of the following 3 behaviors:

a) Immobility – when the rat demonstrated floating behavior with no additional activity other than required to keep its head above the water.
b) Swimming – movement of rat on the water surface throughout the swim chamber, with the rat in a horizontal position.
c) Climbing – upward-directed vigorous thrashing movements with the forepaws, usually along the side of the swim chamber.

**Figure 6.5.1 : Forced swimming test**

**ii) Locomotor activity**

The locomotor activity will be assessed on rats using an actophotometer. Actophotometer operated on photoelectric cells which were connected in circuit with a counter. When the beam of light falling on the photocell will be cut off by the animal, a count will be recorded. These cutoffs will be counted for a period of

10 min and the figure will be taken as a measure of the locomotor activity of the animal. Depending on CNS depressant/stimulant action of the drug, the animals show reduced/increased locomotor activity.


**Figure 6.5.2 : Actophotometer**

**iii) Statistical Analysis**

The means of total immobility time in seconds for FST, behavioral scores (immobility, swimming and climbing counts) in forced swimming test and locomotor activity counts will be analyzed by one-way analysis of variance (ANOVA) further followed by Tukey's multiple comparison test using graph pad prism 6 statistical software. The value of $p < 0.05$ was considered as statistically significant.

# 7. RESULTS

## 7.1 Phytochemical Tests

| COMPOUND | CH |
|---|---|
| Alkaloids | + |
| Flavonoids | + |
| Mucilage | - |
| Tannins | + |
| Gums | - |
| Glycosides | + |
| Non reducing (starch) | - |
| Saponins | - |
| Proteins | - |
| Monosaccharides | - |
| Steroids | + |
| Phenols | - |
| Amino acids | - |
| Carbohydrate | - |
| Reducing sugars | - |

**Table 7.1.1 : Phytochemical test results for CHEE**

Based on the result, it can be confirmed that alkaloids, flavonoids, tannins, glycosides and steroids is present in the *Citrus hystrix DC* leaves. Thus, it is confirmed that *Citrus hystrix DC* contains flavonoids.

# 7. RESULTS

## 7.1 Antidepressant Activity

### 7.1.1 Effect of CHHE and fluoxetine treatment on immobility time in FST.

The effects of CHEE 100, 200 and 400 mg/kg/p.o and fluoxetine 20 mg/kg/p.o treatments on mean immobility time in FST are is shown in the table 7.1.1 and represented graphically in Fig 7.1.1 Upon by visual observation, in 15 min FST (pretest), rats were very active, vigorously swimming, climbing the wall or diving down in plastic cylinder. At the end of 2-3 min the aforementioned activities subsides, in which rat made only those movements which are necessary to keep its head above the water indicating the characteristic behavior called as immobility. 24 h later on the test date when rats subjected to 6 min test session there is increased immobility time. In FST, CHEE 400 and fluoxetine affected the mean immobility time. However, there were no significant differences when compared to control and standard drug fluoxetine group animals.

| Treatment groups | Immobility time (secs)<br>Mean ± SEM |
|---|---|
| Control | 163 ± 4.359 |
| Fluoxetine (Standard) | 109 ± 9.925 |
| CHEE 100 | 180 ± 18.570 |
| CHEE 200 | 162 ± 15.620 |
| CHEE 400 | 157 ± 27.280 |

All the values are expressed as mean ± SEM (n=5)

**Table 7.1.1: Effect of CHEE and fluoxetine treatment on immobility time in FST.**

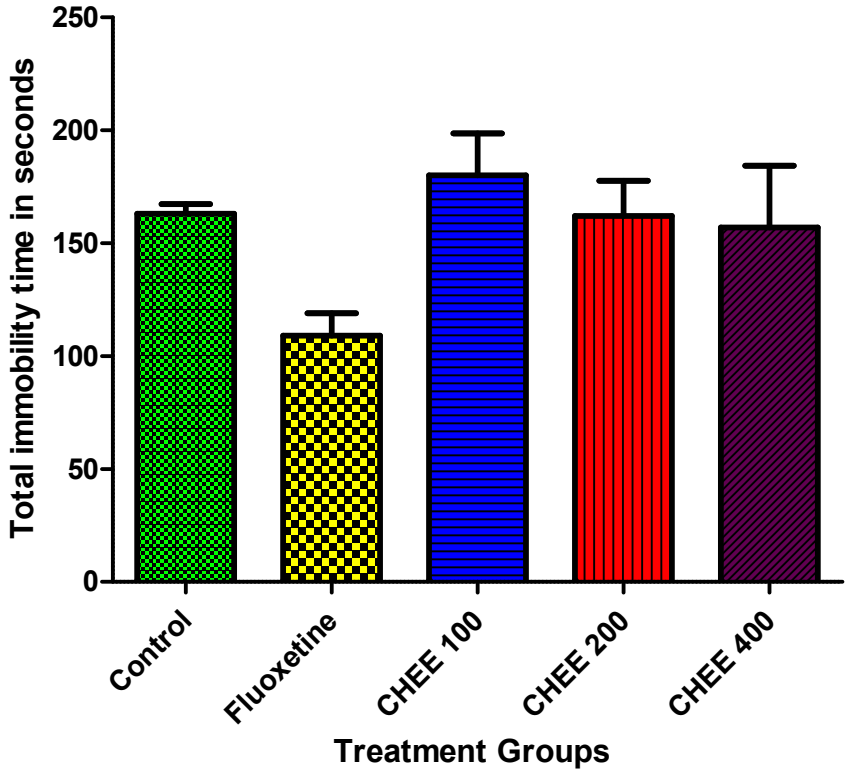



**Fig 7.1.1: Effect of CHEE and fluoxetine treatment on immobility time in FST.**

### 7.1.2 Effect of CHEE and fluoxetine treatment on different behavioral responses.

The effects of CHEE 100, 200 and 400 mg/kg/p.o and fluoxetine 20 mg/kg/p.o treatment in animals on mean scores for the different behavioral responses observed during FST are shown in the table 7.1.2. and represented graphically in Fig 7.1.2

It was found that CHEE and fluoxetine affected scores of immobility, swimming and climbing behaviors. CHEE affected all the behavioural scores at the different doses when compared to control group but was not found to be significant when compared with control group rats.

| Treatment groups | Immobility count | Swimming count | Climbing count |
|---|---|---|---|
| Control | 32.60 ± 0.871 | 20.80 ± 1.855 | 14.80 ± 0.800 |
| Standard | 23.80 ±1.068 | 24.40 ± 2.839 | 25.80 ± 3.184 |
| CHEE 100 | 36.00 ± 3.715 | 19.20 ± 4.716 | 20.40 ± 7.104 |
| CHEE 200 | 32.40 ± 3.124 | 24.40 ± 1.833 | 25.80 ± 5.314 |
| CHEE 400 | 31.40 ± 5.455 | 25.80 ± 3.007 | 36.20 ± 7.228 |

All the values are expressed as mean ± SEM (n=5)

**Table 7.1.2: Effect of CHEEE and fluoxetine treatment on different behavioral responses**

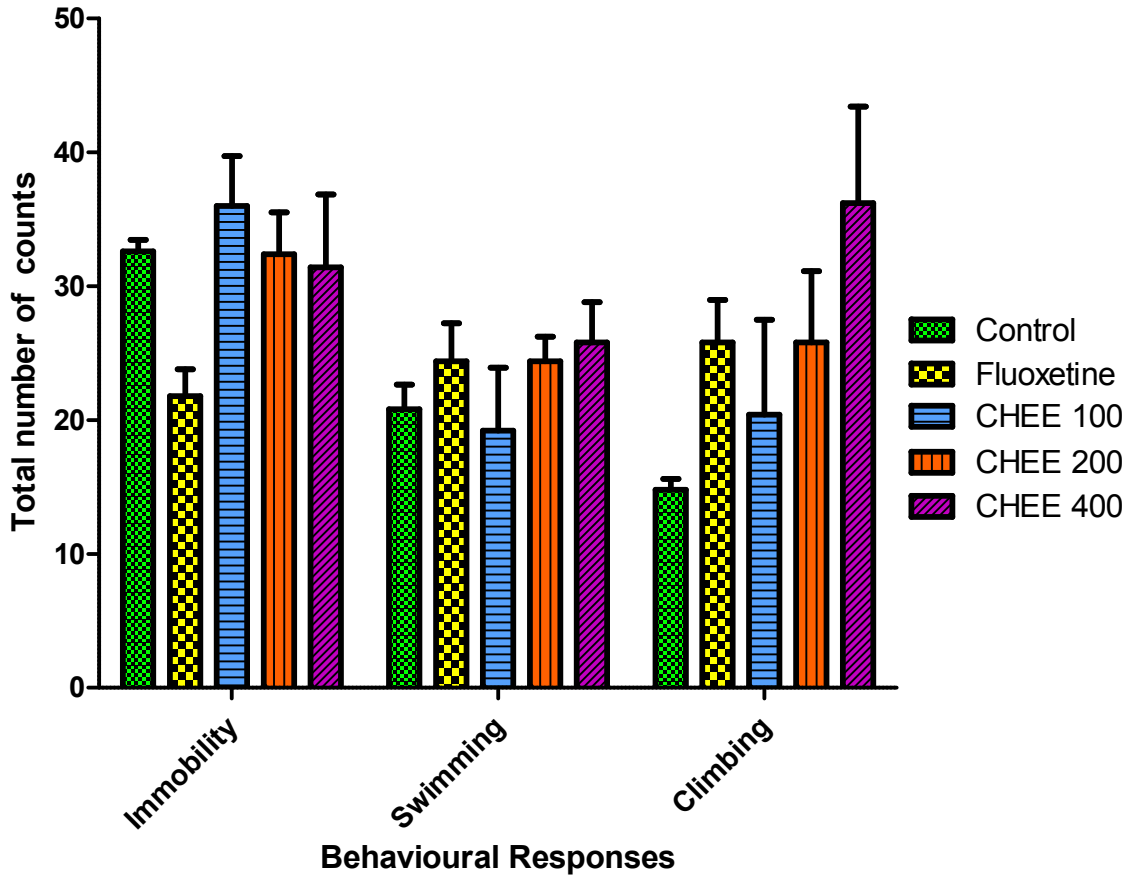



**Fig 7.1.2: Effect of CHEEE and fluoxetine treatment on different behavioral responses**

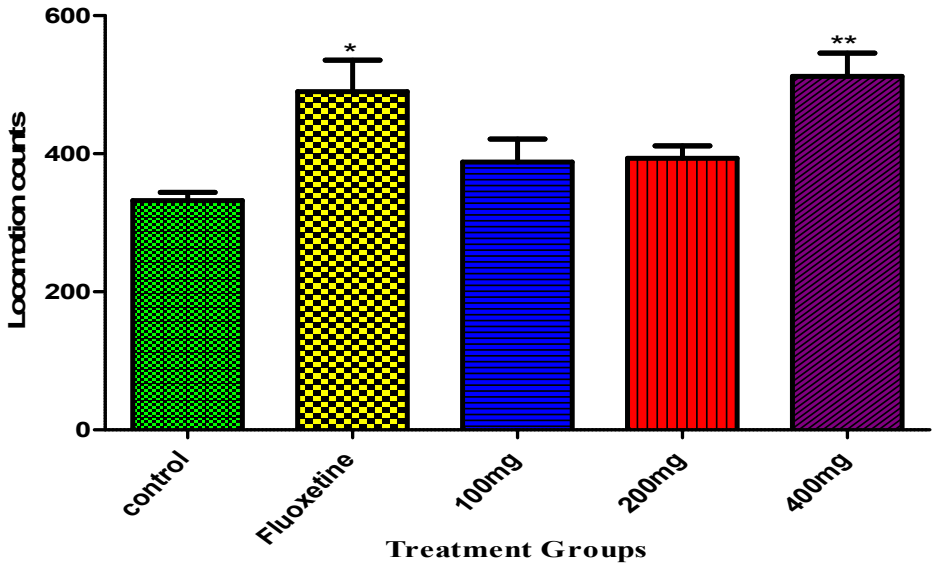
**7.2.1 Effect of CHEE and fluoxetine treatment on locomotor activity in rats measured using actophotometer.**

The mean locomotor counts in CHEE and fluoxetine treated group rats are shown in the table 7.2.1. and represented graphically in Fig 7.2.1 CHEE at the dose of 400 mg/kg/po and fluoxetine at 20 mg/kg/po affected the locomotor activity significantly when compared to control. CHEE 400 significantly ($P<0.01$) and fluoxetine ($P<0.05$) affected the locomotor activity when compared to control rats.

| Treatment groups | Locomotor counts |
|---|---|
| Control | 332 ± 12.00 |
| Fluoxetine (Standard) | 490 ± 45.50* |
| CHEE 100 | 388 ± 33.23 |
| CHEE 200 | 393 ± 18.04 |
| CHEE 400 | 512 ± 33.67** |

***p*<0.01 and **p*<0.05 when compared with control, values are represented as mean ± SEM (n=5)

**Table 7.2.1: Effect of CHEE and fluoxetine treatment on locomotor activity.**



**$p<0.01$ and *$p<0.05$ when compared with control, values are represented as mean ± SEM (n=5)

**Fig. 7.2.1: Effect of CHEE and fluoxetine treatment on locomotor activity.**

## 8. DISCUSSION

The powdered leaves of *Citrus hystrixDC*were extracted using absolute ethanol with the help of Soxhlet apparatus. During each cycle, the non-volatile portion is dissolved in the solvent. After many cycles, the desire component is collected in distillation flask. [26] The methods is using as it is able to dissolve partially soluble components in the solid phase to the liquid phase during the extraction process. Ethanol is used during the extraction because of its ability to extract both polar and non-polar material. Besides, it has a boiling point of 70 °C, which is low enough to separate from the solute. [27]It is essential to separate the ethanol from the solute product as it may cause CNS depression. Nevertheless, ethanol is less harmful to the mammalian cells. Another technique that is used in extraction is maceration. Maceration needed for a period of 7 days. During the maceration, the conical flask is continuously shaken in the incubation chamber because it is needed to remove the saturated layer around the leaves contents with the fresh solvent. Filtration at the end is necessary to separate the insoluble cell contents. [28]

The phytochemicals like carbohydrates, glycosides, alkaloids, flavonoids, steroids and tannins are found to be present in the ethanolic extract of Citrus Hystrix. Among these, flavonoid is a must component in this research project. The presence of flavonoids helps in combating depression in many ways.

Antidepressant activity is determined by forced swim test (FST). Forced swim test is the most widely used method to study the behavioral in depressive-like rodents. The test has high predictive validity for wide range of antidepressant drug and can differentiate from other drugs that are not aim for antidepressant such as anti-anxiety. The active behavior from this test is climbing and swimming as a result of reducing stress. The passive behavior from this test is immobility, which can be seem as preserved energy for the next escape.

Prior to the FST in the period of 24 hours, a 15 minutes pretest is given to the rats to expose the stress to the rat which is important for the tendency to

depression in human. After 24 hours, in the FST, the rats are exposed to 6 minutes test session. Swimming behavior is due to increase in serotonergic neurotransmission while climbing behavior (struggling) is due to increase in catacholaminergic neurotransmission. [29]

This helps us to differentiate the neurochemical mechanism that is causing these behaviors in this test. Another point to be mentioned is the immobility activity in FST is due to adaptive action not because of depressive activity. [30]

The effects of CHEE 100, 200 and 400 mg/kg/p.o and fluoxetine 20 mg/kg/p.o treatments in animals on mean scores for the different behavioral responses were observed during FST. It was found that CHEE and fluoxetine affected scores of immobility, swimming and climbing behaviors. CHEE affected all the behavioral scores at the different doses when compared to control group but was not found to be significant when compared with control group rats. It is important to note that the effect of immobility is not effective in treating depression. Another test is needed to rule out the basal activity level that is not determining factor in this test. [29]

Locomotor activity is an index of alertness in the CNS depressant activity. When the animal cut off the photocell, the movement is recorded digitally. CHEE at the dose of 400 mg/kg/p.o and fluoxetine at 20 mg/kg/p.o affected the locomotor activity significantly when compared to control, indicating it may stimulate CNS.

Lastly, even though the finding in the FST is not significant, CHEE is showing increase in climbing and swimming activity when the doses increase from 100 to 200 and 400 mg/kg/p.o respectively. In the locomotor activity study, there is a significant finding that can be suggested that the CHEE is effective in stimulating CNS. Moreover flavonoid present in CHEE may have shown the potential in combating for depression and stimulating CNS. [31]

## 9. CONCLUSION

The present study demonstrated CHEE to show antidepressant like activity and CNS stimulant effect. This suggests its action on CNS which may contribute in the management of depression. Thereby, we conclude that CHEE may contribute in the management of CNS depression disorder. However, further details studies are suggested for understanding Citrus hystrix *DC*leaves ethanolic extract's complete pharmacology in understanding its mechanism in CNS.

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# 11. APPENDICES

1. WHO: World Health Organization

2. DSM-V: The Diagnostic and Statistical Manual of Mental Disorder, Fifth Edition

3. PMDD: Premenstrual Dysphoric Disorder

4. PMS : Premenstrual Syndrome

5. 5-HTTLPR : Serotonin-transport-linked polymorphic region

6. 5-HT: 5_Hydroxytrptamine

7. HPA : Hypothalamic-Pituitary-Adrenal

8. BNDF: Brain-derived neurotrophic factor

9. HC: Habenular Comissure

10. D1 receptor : Dopamine Receptor

11. CSF: Cerebrospinal fluid

12. REM: Rapid eye movement

13. OCD : Obsessive Compulsive Disorder

14. NE : Norepinephrone

15. DA: Dopamine

16. 5HTA1: 5-Hydroxytryptamine receptor 1A

17. 5HT2C: 5-Hydroxytryptamine receptor 2C

18. cCBT:Computerised cognitive behavior therapy

19. CBT: Cognitive behavior Therapy

20. BAT: Behavioral approach

21. PT: Physical therapy

22. BCT: Behavioral couples therapy

23. PST: Psychological skills training

24. BDT: Dialectical behavior therapy

25. SUP: Supportive Counseling

26. ECT: Electroconvulsive therapy

27. DLGG: Diffuse low-grade glioma

28. LPGG: Lysophosphatidylgycerol

29. TPA: Tissue plasminogen activator

30. MeOH : Methanol

31. ACHE: Acetylcholinesterase

32. MAOK: Mitogen-activated protein kinase

33. P13K/Akt: Phosphoinositide-3=kinase/protein kinase B

34. BBB: Blood brain barrier

35. FST: Forced swimming test

36. ANOVA: Analysis of variance

37. SD: Sprague-Dawley