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Original Research Article

Pharmacological Screening of *Calycatome villosa* Methanolic Extract Growing Wild in Libya Using Albino Mice

Sumaya O. Aljabri[1*], Suhera M. Aburawi[1], Feras W. Alkayed[2], Nisrin A. Alrbige[1], Nada E. Akordi[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, University of Tripoli, Libya
[2]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, University of Tripoli, Libya

***Corresponding Author:** Sumaya O. Aljabri
Department of Pharmacognosy, Faculty of Pharmacy, University of Tripoli, Libya

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Abstract: Depression is a common mental disorder, causing disability worldwide and contributing to the overall global burden of disease. Antidepressant drug treatment takes several weeks to produce an effect; this effect is often accompanied by unwanted side effects. Therefore, the use of a crude form of herbal extracts could be an alternative therapy for depressive disorders. ***Aims of the Work:*** To investigate the phytochemical composition of aerial parts (flowers and leaves) *Calicatome villosa* extract; also to evaluate the neurobehavioral effect on the nervous system by applying the Irwin test, and to screen for the antianxiety and antidepressant-like activity using pulse maze and forced swimming maze**.** ***Material and Methods:*** The fresh aerial parts of *calycatome villosa* were collected in March 2018 from Alkums city -Libya. Preliminary Phytochemical analyses were carried out on the methanolic extract. Screening for the presence of various phytochemical compounds like; alkaloids, carbohydrates, tannins, phytosterols, saponins, flavonoids, and proteins were carried out. ***Conclusion*****:** Preliminary phytochemical screening of *Calycatome villosa* methanolic extract revealed the presence of alkaloids, saponins, carbohydrates, phytosterols, phenols, and tannins. The pharmacological screening of the extract showed antidepressant, antipsychotic, CNS depression, and Muscle relaxant effects. The extract showed CNS depression using the Irwin test, while the spontaneous motor activity was not changed using EPM. *Calycatome villosa* methanolic extract with the dose used did not show an antianxiety-like effect using EPM.

Keywords: Depression, *Calycatome villosa*, Antidepressant activity, Anxiolytic activity, preliminary phytochemical screening, Irwin blind test, Elevated plus-maze test, forced swimming maze test.

INTRODUCTION

Depression is a common mental disorder. It is a leading cause of disability worldwide and is a major contributor to the overall global burden of disease. More women are affected by depression than men. Depression can lead to suicide. Depression may become a serious health condition. It can cause the affected person to suffer greatly and function poorly at work, at school, and in the family. At its worst, depression can lead to suicide (WHO, 2021).

Antidepressant drug targets and systems dysregulated in depressed states include the hypothalamic–pituitary–adrenal (HPA) axis, the monoaminergic system, and the γ-aminobutyric acid (GABA) system, and adult hippocampal neurogenesis (Taylor *et al.*, 2005).

Clinically, not all patients respond to antidepressant treatment, the therapeutic effects take several weeks to manifest and these effects are often accompanied by unwanted side effects (Taylor *et al.*, 2005). Therefore, the use of herbal extracts in their crude form or a semi-purified form is gaining ground among clinicians as well as patients as an alternative therapy for depressive disorders [Guan and Liu, 2016; Fajemiroye, 2016].

Herbal therapy has been practiced for many years, and their importance has been recognized globally. The natural product compounds discovered from medicinal plants have provided numerous clinically important drugs, which are being used for treating different diseases (Oyedepo and Palai, 2021). The isolation of bioactive compounds/secondary metabolites leads to discover new drugs with interesting pharmacological properties. Studies are required to assess the safety profiles of such herbal products and with the appropriate animal protocols (Tsatsakis *et al.*, 2021).

El-Guenduol (Calycatome villosa) plant belongs to the Fabaceae family; it is one of the most widespread plants in Libya. There are very limited studies, especially in terms of its biological effects on the central nervous system; therefore, this plant was chosen to be the subject of this study.

Previous studies showed that Calicatome villosa has Antibacterial, Antioxidant (Chikhi *et al.*, 2014; Elkhamilichi *et al*., 2017) and diuretic and vasodilator activities (Cherkaoui *et al*., 2008). Calycotome villosa (C. villosa) is a hairy perennial with yellow flower heads in branched clusters; however, no uses in folk medicine are known for this species. Phytochemical studies have revealed that the plant is rich in flavonoids, terpenes, alkaloids, falcarinol, and anthraquinones (Chikhi *et al.*, 2014).

Aims of the Work

This study aims to investigate the phytochemical composition of Arial parts (flowers and leaves) *Calicatome villosa* extract; also to evaluate the neurobehavioral effect on the nervous system by applying the Irwin test, and to screen for the antianxiety and antidepressant-like activity using pulse maze and forced swimming maze**.**

MATERIAL AND METHODS

Plant Material

In the present study, the fresh aerial parts (stems, leaves, and flowers) of *calycatome villosa* were used, the plant was collected in March 2018 from Alkums city -Libya. The plant material was authenticated and identified by botanists in the herbarium of the faculty of sciences, Tripoli University. The plant was dried in shade for about two weeks until complete drying.

Preparation of Methanolic Extract

Air-dried plant material was ground and subjected to extraction by soxhlate apparatus, the obtained methanolic extract was then filtered and the filtrate was evaporated using a rotary evaporator at 45 °c. The concentrated extract was stored in closed jars for further analysis.

Preliminary Phytochemical Analysis

The methanolic extract was screened for the presence of various phytochemical compounds like; alkaloids, carbohydrates, tannins, phytosterols, saponins, flavonoids, and proteins. Phytochemical screening was carried out according to the standard methods (Tiwari *et al.*, 2011).

1-Detection of Alkaloids:

The extract was dissolved in dilute hydrochloric acid and filtered.

- **Mayer's Test:** The filtrate was treated with Mayer's reagent (potassium mercuric iodide). The formation of a yellow-colored precipitate indicates the presence of alkaloids.
- **Wagner's Test:** The filtrate was treated with Wagner's reagent (iodine in potassium iodide). The formation of brown/reddish precipitate indicates the presence of alkaloids.
- **Dragendroff's Test:** The filtrate was treated with Dragendroff's reagent (solution of potassium bismuth iodide). The formation of a red precipitate indicates the presence of alkaloids.

2- Detection of Carbohydrates:

Extract dissolved in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.

- **Fehling's Test:** The filtrate was hydrolyzed with dil. HCL, neutralized with alkali and heated with Fehling's A and B solutions. The formation of a red precipitate indicates the presence of reducing sugars.

3-Detection of Saponins

- **Foam Test:** 0.5 gm of the extract was shaken with 2 ml of water. If the foam produced persists for ten minutes, it indicates the presence of saponins.

4-Detection of Phytosterols

- **Salkowskis Test:** The extract was treated with chloroform, then filtered. The filtrates were treated with a few drops of conc. Sulphuric acid is shaken and allowed to stand. The appearance of golden yellow color indicates the presence of triterpenes.

5-Detection of Phenols

- **Ferric Chloride Test:** The extract was treated with 3-4 drops of ferric chloride solution. The formation of bluish-black color indicates the presence of phenol.

6-Detection of Tannins:

The extract was treated with a few drops of 1% $Fecl_3$ solution. The formation of brownish green color indicates the presence of tannins.

7-Detection of flavonoids

- **Alkaline Reagent Test:** The extract was treated with a few drops of sodium hydroxide solution. The formation of intense yellow color, which becomes colorless with the addition of diluted acid, indicates the presence of flavonoids.

8-Detection of Proteins

- **Xanthoproteic Test:** The extract was treated with a few drops of conc. Nitric acid. The formation of yellow color indicates the presence of proteins.

1. Preliminary Neuropharmacological Screening

a. Irwin Test

The neuropharmacological Profile study suggested by Irwin provides clues for the classification of the active compounds, to proceed for further testing. In this procedure, mice after administering the drugs are observed for the neuropharmacological profile, which is divided into behavioral, neurological, and autonomic changes after administration of increasing doses of drugs (Moser, 2010).

b. Anxiety Screening

Elevated Plus- Maze Test

Elevated plus-maze is composed of two open arms (30*5cm) and two close arms (30*5*15cm) that extended from a common central platform (5*5cm). The apparatus was elevated to a height of 45 cm above floor level (Vinader-Caerols *et al.,* 2006). Mice were gently handled by the right hand and placed on the center squire of the maze facing into the close arm.

The different parameters were scored to evaluate the anxiolytic effect and spontaneous motor activity in the elevated plus-maze, including time spent by the mouse in each of the arms, lines crossed in close or open arms, and the number of entries into close or open arms. An arm entry was defined as the entry of all four paws into the arm (Kumar and Sharma, 2005). The total line crossed, and total number of entries was calculated. The total line crossed and the total arm entries (Rodgeres *et al.,* 1997; Aburawi, 1999) express the spontaneous motor activity. Anxiety measures were calculated by the time spent in the close arm by the total time of the test (Aburawi, 1999). The duration of the test was 4 minutes.

c. Acute antidepressant Test

Forced Swimming Maze Test

Mice were placed individually in glass cylinders (height 27 cm, diameter 15 cm) filled with water to a height of 16 cm (maintained at 23-25˚C). The duration of the test was 6 minutes. The time of the two behavior parameters (duration of immobility and duration of climbing) was recorded during the last 4 min of the 6 min testing period (Rojecky *et al.,* 2004). Immobility behavior is defined as the animal floating on the surface with front paws together and making only those movements with the hind limb, which were necessary to keep afloat. Climbing behavior is defined as upward-directed movements of fore paws along the side of the swim chamber (Cryan *et al.,* 2002).

Statistical Analysis

Descriptive statistical analyses were performed using the computer program SPSS (software package, version 10); to verify whether the data were normally distributed using the Kolmogrove-Simirnove test maximum deviation test for goodness of fit. If the parameters were parametric, treatments were compared by one-way ANOVA; a Post-Hoc test (LSD and Duncan test) was applied. If the parameters were nonparametric, treatments were compared by the Mann-Whitney *U* test for an unmatched sample. The differences were considered significant at the p-value ≤ 0.05. The values are expressed as the mean standard error.

RESULTS

- ***Preliminary Phytochemical Screening***

Preliminary phytochemical screening revealed the presence of alkaloids, saponins, carbohydrates, phytosterols, phenols, and tannins (Table 1).

Table 1: Phytochemical constituents of *C. villosa*

Phytochemical	Test	Methanol	Dichloromethane	Hexane
Alkaloids	Mayer's	++	+	-
	Wagner's	++	+	-
	Dragendroff's	-	-	-
Carbohydrates	Fehling's	++	++	++
Saponins	Froth	+	-	-
	Foam			
Phytosterols	Salkowskis	+	-	-
Phenols	Ferric chloride	+	+	-
Tannins	Ferric chloride	+	+	-
Flavononoids	Alkaline reagent	-	-	-
Proteins	Xanthoproteic	-	-	-

Irwin Blind Test

Irwin test application showed that the passivity and motor incoordination (abnormal gait) were increased; while the body posture and limb position were decreased compared to the control (Table 2).

Table 2: Irwin blind test on *Calicotome villosa* methanolic extract using albino mice

		Basic score	Control 1	Control 2	Ex. 250 3	Ex. 250 4	Ex. 500 5	Ex. 500 6	Suggestion
Behavioral Awareness	Passivity	0	0	0	2	2	2	2	Antipsychotic CNS depression Muscle relaxant
Posture	Body posture	4	4	4	3	3	2	2	Neuromuscular blockade Central disturbances
	Limb position	4	4	4	2	2	1	1	Neuromuscular blockade Central disturbances
Motor incardination	Abnormal gait	0	0	0	2	2	3	3	Muscular relaxation

- *Calicotome villosa extract screening for antianxiety and spontaneous motor activity like action using plus maze in albino mice*
- *Calicotome villosa extract screening for antianxiety like action using plus maze in albino mice*

Administration of methanolic extract, of 250 and 500 mg/kg, did not change the anxiety measure ($p = 0.440$ and $p = 0.492$, respectively), while diazepam decrease significantly the anxiety measure at $p = 0.004$ (table 3, figure 1).

Table 3: *Calicotome villosa* methanolic extract screening for antianxiety-like action applying plus maze in albino mice

Treatments	Anxiety measure	P compared to control	P compared to Extract (500mg/kg)
Control T80 (1%T80)	0.995 ± 0.0026		
Diazepam (1 mg/kg)	0.877 ± 0.0205	0.004	
Extract (250 mg/kg)	0.956 ± 0.0236	0.440	0.797
Extract (500 mg/kg)	0.968 ± 0.0247	0.492	

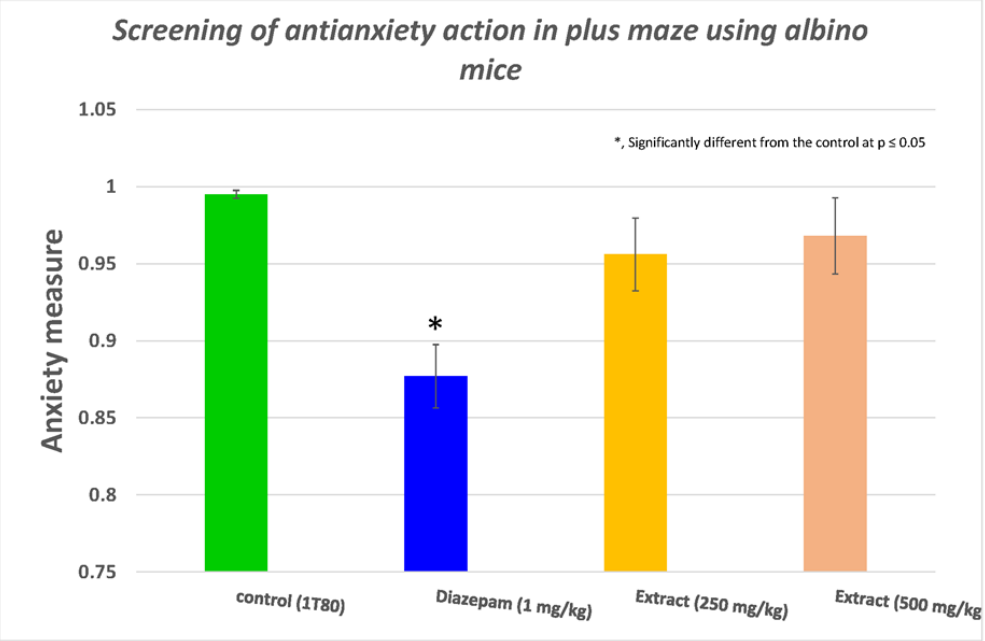

Figure 1: Screening of antianxiety in plus maze using albino mice

• ***Calicotome Villosa Extract Screening Effect on the Total Number of Entries Using Plus Maze in Albino Mice***

Administration of *Calicotome villosa* methanolic extract, with the doses of 250 and 500 mg/kg, or even the administration of diazepam did not change the total number of entries produced by the mice at p = 0.459, 0.926, and 0.864 respectively (table 4, figure 2).

Table 4: ***Calicotome villosa extract screening effect the total number of entries using plus maze in albino mice.***

Treatments	Total number of entries	P compared to control	P compared to Extract (500mg/kg)
Control T80 (1%T80)	2.67 ± 0.843		
Diazepam (1 mg/kg)	4.00 ± 1.592	0.459	
Extract (250 mg/kg)	2.50 ± 0.957	0.926	0.932
Extract (500 mg/kg)	3.00 ± 1.438	0.864	

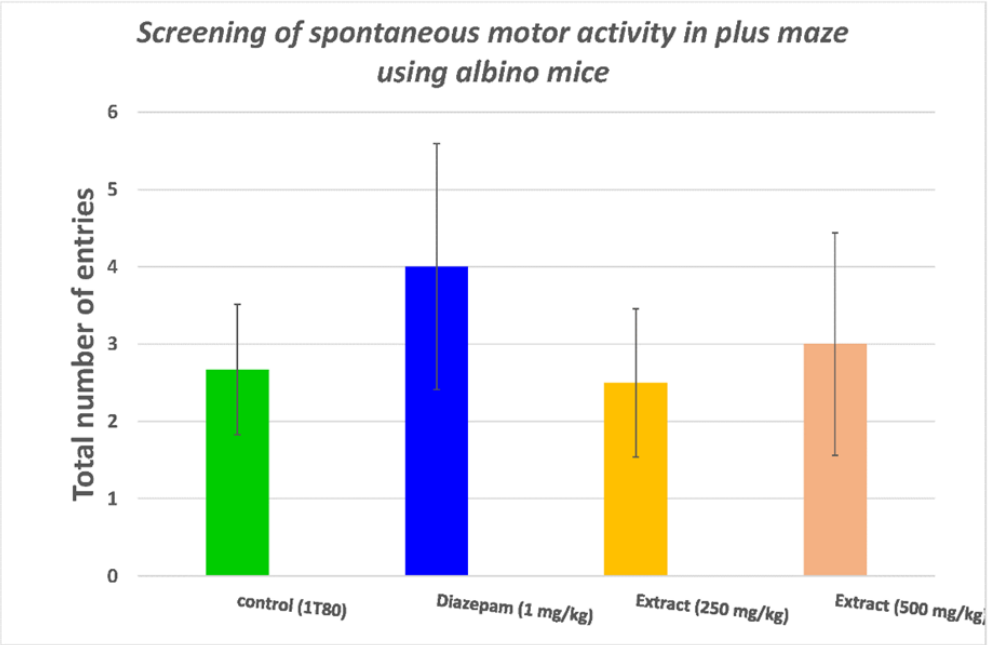

Figure 2: Screening of spontanous motor activity in plus maze using albino mice

• ***Calicotome Villosa Extract Screening the Effect on the Total Lines Crossed Using Plus Maze in Albino Mice***

Administration of *Calicotome villosa* methanolic extract or even the administration of diazepam did not change the total lines crossed by the mice at $p > 0.05$ (table 5, figure 3).

Table 5: ***Calicotome villosa extract screening the effect on the total lines crossed using plus maze in albino mice.***

Treatments	Total lines crossed	P compared to control	P compared to Extract (500mg/kg)
Control T80 (1%T80)	15.8 ± 4.30		
Diazepam (1 mg/kg)	28.0 ± 7.33	0.166	
Extract (250 mg/kg)	13.8 ± 5.35	0.815	0.614
Extract (500 mg/kg)	18.1 ± 6.48	0.785	

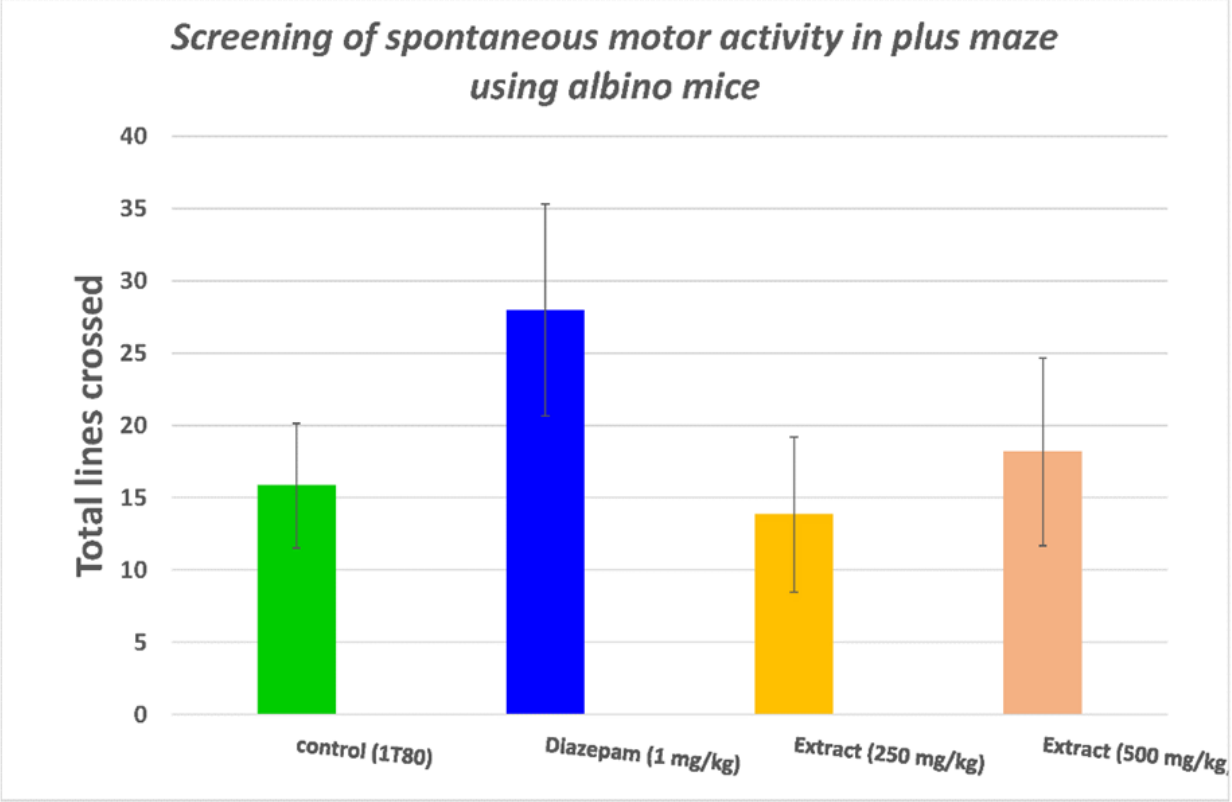

Figure 3: Screening of spontaneous motor activity in plus maze using albino mice

• ***Calicotome Villosa* Methanolic Extract Screening for Antidepressant-Like Activity Using Forced Swimming maze in Albino Mice**

Imipramine and methanolic extract in a dose of 500 mg/kg produced a significant decrease in the duration of immobility produced by mice ($p = 0.006$, 0.002, respectively); while methanolic extract at a dose of 250 mg/kg showed an insignificant decrease in the duration of immobility ($p = 0.114$). Administration of methanolic extract at a dose of 500 mg/kg decreased the duration of immobility significantly compared to methanolic extract with a dose of 250 mg/kg, at $p = 0.037$ (table 6, figure 4).

***Table 6: Calicotome villosa* extract methanolic screening for antidepressant like activity using a forced swimming maze in albino mice**

Treatments	Duration of immobility (seconds)	P compared to control	P compared to Extract (500mg/kg)
Control T80 (1%T80)	151.0 ± 10.28		
Imipramine (10mg/kg)	73.2 ± 25.84	0.006	
Extract (250 mg/kg)	109.2 ± 18.61	0.114	0.037
Extract (500 mg/kg)	60.9 ± 12.33	0.002	

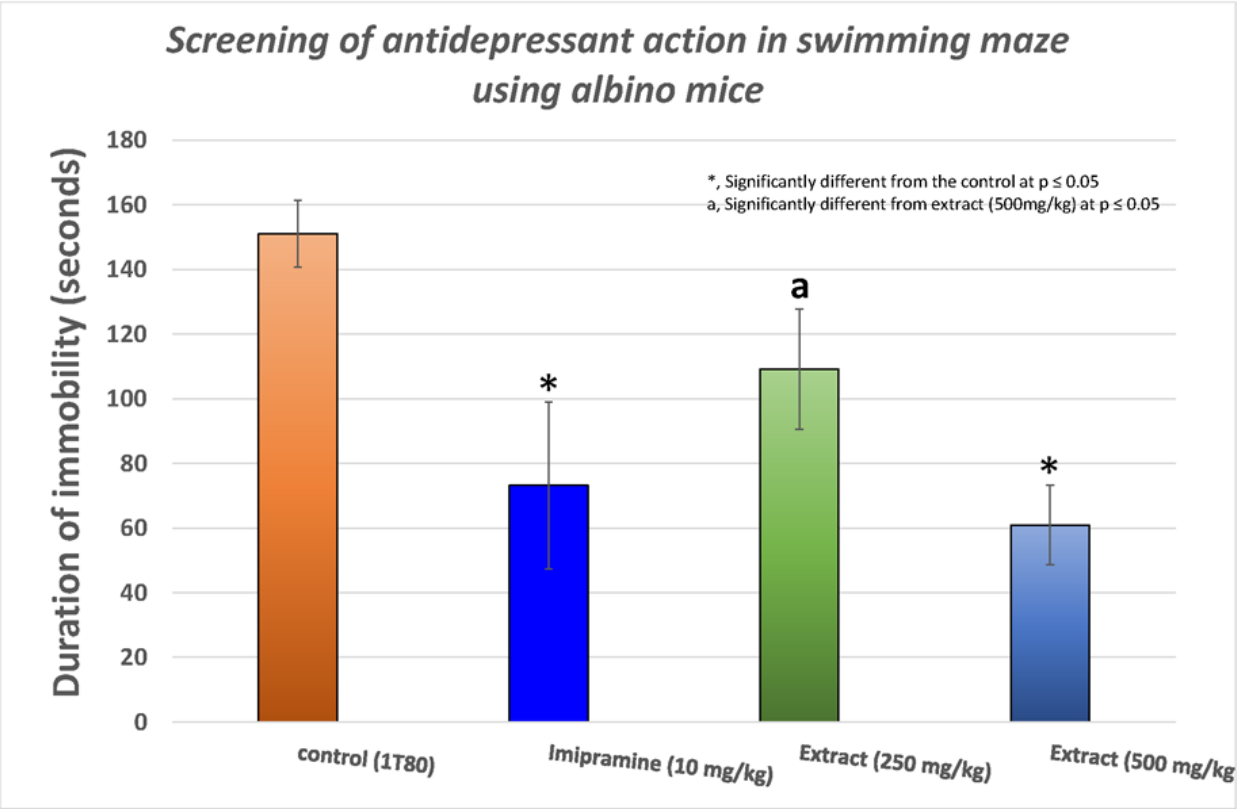

Figure 4: Screening of antidepressant action in swimming maze using albino mice

DISCUSSION

Natural products have gained popularity for promoting healthcare, or disease prevention (Yan *et al.,* 2020). These products are considered rich sources of potential new drugs (Munir *et al.,* 2020). Natural products have a wide range of pharmacological or biological activities (Harvey *et al.,* 2010). Extensive research on natural compounds of plant origin was applied, to find a better and safer alternative treatment for health disorders (Esra *et al.,* 2021). The herbal products for the management of psychiatric disorders have gained importance (Munir *et al.,* 2020). The phytochemical compounds have pharmacological roles in treating various disease conditions, and widely available in nature, and are commercially beneficial (Yan *et al.,* 2020).

Plants used in traditional medicine contain compounds in their secondary metabolism (Lundstrom *et al.,* 2017); these compounds as alkaloids, phenols, sterols, carbohydrates, tannins, terpenes, and phytoalexins, all may have important biological activities (Arnason *et al.,* 2005; Bérdy, 2005). Studies showed that these phytochemicals have significant potential in in-vitro and in-vivo models of psychiatric disorders (García-Ríos *et al.,* 2020, Esra *et al.,* 2021). These metabolites have shown anxiolytic and antidepressant effects in various experimental models (García-Ríos *et al.,* 2020).

In this work, the active constituents evaluated in *Calicotome villosa* methanolic extract are alkaloids, carbohydrates, saponins, phytosterols, phenol, and tannin. These active constituents showed antidepressant, muscle relaxant, CNS depressant, and antipsychotic-like activity. The extract did not show antianxiety-like activity when using EPM.

Antidepressant of Calicotome Villosa Methanolic Extract

Mental depression is a common global mental problem that affects people. Depressant patients suffer an inability to experience interest and pleasure, self-doubt, loss of concentration, social anxiety, appetite, and sleep disorder (McCarter, 2008).

Preclinical studies demonstrated that alkaloids have powerful antidepressant effects, but their mechanisms of action are still unclear. Preclinical studies are needed to evaluate their potency, efficacy, and safety before they can be incorporated into clinical practice (García-Ríos *et al.,* 2020).

Alkaloids are naturally consisting components of carbon, hydrogen, oxygen, and nitrogen molecules (Harvey *et al.,* 2010; they have pharmacological effects and are used as medications; it has antidepressant-like activity (Perviz *et al.,* 2016; Kumarnsit *et al.,* 2007). Using the forced swim test and tail suspension test, alkaloids produce antidepressant action without any significant effect on locomotor activity (Idayu *et al.,* 2011). These alkaloids have shown neuroprotective activity, makes them a choice for the treatment of depressive disorders (Splettstoesser *et al.,* 2005; Fortunato *et al.,* 2009).

The indole moiety of indole alkaloids contains two oxygen atoms instead of nitrogen atoms; this structure may facilitate the antidepressant effect (Munir *et al.,* 2020). Indole alkaloids demonstrated a broad spectrum of pharmacological properties (Cao *et al.,* 2007). They may interact with GABA receptors inside the brain via GABA-aminotransferase inhibition, interference in intake and uptake of functional GABA within synaptosomes, and interaction with benzodiazepine/GABA receptors, leading to antidepressant effect; (García-Ríos *et al.,* 2020; Cao *et al.,* 2007; Sichardt *et al.,* 2007). The alkaloid derivatives of the β-carbolines showed antidepressant effects in mice, dose-dependently; authors suggested that the effect may occur through an inverse agonist mechanism of the benzodiazepine receptors (Farzin and Mansouri, 2006).

By interacting with the hypothalamic-pituitary-adrenal (HTPA) axis within the endocrine system, Mitragynine alkaloid significantly reduced the release of corticosterone in mice exposed to FST and TST (Idayu *et al.,* 2011). While punarnavine alkaloid showed antidepressant-like activity in unstressed and stressed mice by a decrease in plasma nitrite levels and due to its antioxidant activity and through a decrease in plasma corticosterone levels (Dhingra *et al.,* 2014). Evodiamine alkaloid produced an antidepressant-like effect in a rat model of chronic unpredictable mild stress; Evodiamine treatments ameliorated the corticosterone hypersecretion induced by the model (Jiang *et al.,* 2015).

Alkaloids such as β-carboline inhibit monoamine reuptake systems and inhibit monoamine oxidase (Cao *et al.,* 2007; Fortunato *et al.,* 2009; Splettstoesser *et al.,* 2005; Herraiz and Guillén, 2011) leading to increase monoamines levels in the synapse, producing antidepressant-like activity. Alkaloids as barettin have structural similarities to serotonin, therefore may facilitate the function of 5HTR in case of 5-HTR disorders (Hedner, *et al.,* 2006) and interact with 5HT. Administration of 1-methyl-beta-carboline alkaloids into the hippocampus of rats, increased the concentration of 5-HT (Adell *et al.,* 1996); leading to antidepressant activity. It was found that Evodiamine- alkaloid administration produced an antidepressant-like effect through an increase of 5-HT and NA levels in the hippocampus (Jiang *et al.,* 2015). Another study showed that alkaloids may bind to type 5-HT_{2A} serotonergic receptors but shows no affinity to dopaminergic or BZ receptors (Glennon *et al.,* 2000). Several studies have suggested that dopaminergic receptors are involved in the antipsychotic, and antidepressant activities of kratom main alkaloid (mitragynine) (Hanapi *et al.,* 2021). The antidepressant effect of psychotropic β-carboline alkaloids is due to the high affinity for 5-hydroxytryptamine, dopamine, benzodiazepine, and imidazoline receptors and the stimulation of locus coeruleus neurons (Yu *et al.,* 2003).

Some Harmala alkaloids have neuroprotective effects in the nervous system, through several sites of action at the cellular level of benzodiazepine receptors, 5-HT and GABA(A) receptors have been identified. It was concluded also that harmaline and harmane are modulators of I(Ca(V)) in vitro. It was mainly due to a reduction in the sustained calcium channel current (I(Ca(L+N))), while the transient voltage-gated calcium channel current (I(Ca(T))) was only partially affected. This might be related to their neuroprotective effects (Splettstoesser *et al.,* 2005).

Using FST in rats, β-carboline harmine alkaloids produced antidepressant-like effects, it was explained that alkaloids increased brain-derived neurotrophic factor (BDNF) protein levels and expression in the hippocampus of rats (Fortunato *et al.,* 2010; Jiang *et al.,* 2015). Chronic unpredictable mild stress produced corticosterone hypersecretion, causing depression-like behavior in rats; evodiamine alkaloids decreased serum corticosterone levels and increased 5-HT and NA levels (Jiang *et al.,* 2015).

Caffeine alkaloid is a non-selective adenosine antagonist for A_1/A_{2A} receptors and has been demonstrated to modulate behavior in classical animal models of depression. Caffeine alkaloids can improve depression in humans, as well as in animal models. Authors concluded that caffeine alkaloids could be therapeutic agents for the treatment of depression (López-Cruz *et al.,* 2018).

Adenosine receptors have a role in mood and anxiety disorders. Activation of A_{2A} receptors is associated with increased depression-like symptoms (Calker *et al.,* 2019), and associated with a decrease in synaptic plasticity, a reduced density of synaptic proteins, also an increase of A_{2A} receptors in the striatum and in glutamatergic terminals in the hippocampus (Crema *et al.,* 2013; Kaster *et al.,* 2015). While increased A_1 receptor signaling elicits rapid antidepressant effects (Calker *et al.,* 2019).

There is evidence that A_2 and A1 receptors are expressed in the hippocampus (Cunha *et al.,* 1994). Furthermore, A_{2A} receptor antagonists evoke antidepressant-like effects in the forced swim test and the tail suspension test in rodents (Hodgson *et al.,* 2009; Yamada *et al.,* 2013). Activation of A_1 and inhibition of A_{2A} receptors elicit antidepressant effects. The antidepressant effects of activation of A_1 receptor signaling occur through an increase of signaling via Homer1a which leads finally to a modulation of AMPA receptor functioning (Holz *et al.,* 2019); where Homer1a switches mGluR5 signaling to increase AMPA receptor activity for the rapid antidepressant actions (Albert, 2019).

One possible explanation for the antidepressant-like effects of caffeine alkaloids as A_{2A} antagonists is the facilitation of activity of A_1 receptors (Gass *et al.,* 2010). Alkaloids such as caffeine, adenosine antagonist produce antidepressant-like effects via antagonism at adenosine A_{2A} receptors, by selective A_{2A} receptor antagonists, and by A_{2A} receptor deletion in forebrain neurons (Kaster *et al.,* 2015; Lopez-Cruz *et al.,* 2018).

Caffeine alkaloid increases intracellular concentrations of cyclic adenosine monophosphate (cAMP) by inhibiting phosphodiesterase enzymes; increased cAMP could also lead to an increase in blood catecholamine (Daly, 1993; Cappelletti *et al.,* 2015). A study suggested that caffeine alkaloids could be used as an enhancer of antidepressant pharmacotherapy (Kale *et al.,* 2010).

Accumulated evidence that depression is associated with increased activity in the immune system; depression is associated with C-reactive protein (CRP), interleukin (IL)-1, and IL-6 in community and clinical samples (Smith, 1991; Howren *et al.,* 2009). Anti-inflammatory treatment may yield antidepressant properties (Müller *et al.,* 2006; Tyring *et al.,* 2006). It was found that caffeine alkaloid protected against chronic inflammation; as the more caffeine that people consumed, the more protected they were against chronic inflammation. Caffeine alkaloid has been shown to block the effects of adenosine in the brain (Cleveland Heartlab, 2017; Shushtari and Abtahi Froushani, 2017). Caffeine decreased the lipopolysaccharide-induced inflammatory mediator (nitric oxide). Caffeine treatment also reduced the expression of pro-inflammatory genes, including inducible nitric oxide synthase (iNOS), cyclooxygenase-2 (COX-2), interleukin (IL)-3, IL-6 and IL-12. Caffeine inhibited nuclear translocation of nuclear factor κB (NF-κB) via IκBα phosphorylation (Hwang *et al.,* 2016); this translocation can be induced by lipopolysaccharide (Pisetsky, 2014).

Caffeine alkaloid produces directs down-regulation of adenosine A2 receptors to counter the suppressive effect of adenosine on brain dopaminergic transmission (Hussain *et al.,* 2018). Alkaloids may interact with the dopaminergic pathway, and may interact non-competitively with presynaptic dopamine receptors (Marek and Roth, 1980); also, kratom alkaloids produced dose-dependent presynaptic (functional antagonistic) and postsynaptic (agonistic) action at dopamine receptors (Hanapi *et al.,* 2021), inducing antidepressant effect. It was found that alkaloids produce antidepressant activity by inhibiting MAO-A and MAO-B enzymes (Ali *et al.,* 1998; Smith *et al.,* 2013), this mechanism will increase the monoamine ending with an antidepressant-like effect.

Hussain *et al.,* (2018) and Harvey *et al.,* (2010) concluded that Alkaloids has adenosine receptor agonists, anti-oxidants, MAO inhibitors, and dopaminergic and NMDA antagonist. While García-Ríos *et al.,* (2020) concluded that alkaloids and sterols' mechanisms of action are by activation of the critical enzyme for catecholamine synthesis (e.g., tyrosine hydroxylase) or the inhibition of their limiting enzymes, MAO-A and MAO-B, and transporters, leading to the stimulation of the vesicular storage monoamine and the release of neurotransmitters toward the synaptic cleft.

Studies found that saponins have good preventive and therapeutic effects on neurological diseases of the brain (Su *et al.,* 2014), especially in antidepressants (Su *et al.,* 2014; Xiang *et al.,* 2011; Zhang *et al.,* 2016). Saponins may function as an antidepressant in rodents, which may be mediated by modulation of brain monoamine neurotransmitters and intracellular Ca^{2+} concentration. Saponins increase the levels of 5-hydroxytryptamine, dopamine, and noradrenaline; additionally, they reduced intracellular Ca^{2+} in cultured neurons (Xiang *et al.,* 2011). Another study showed that saponins increased the NA levels in the brain regions of mice exposed to FST but did not affect the 5-HT and DA levels. Moreover, saponins treatment could reverse swim stress-induced increased levels of serum ACTH and corticosterone (Zhang *et al.,* 2016).

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In Lee *et al.,* (2012) and Jiang *et al.,* (2012) studies, administration of ginsenoside saponins, in experimental animals, before immobilization stress significantly improved helpless behaviors in the FST model and inhibited the stress-induced behavior; saponins down-regulated serum corticosterone level in the hippocampus leading to the antidepressant effect.

It was found that Saponins inhibited the turnover of the tryptophan-serotonin (5-HT) in the hippocampus, and regulated the secretory activity of microglia cells (Xie *et al.,* 2918). It was also found that serotonin (5-HT) and noradrenaline (NE) levels in the hippocampus and frontal cortex were significantly increased in saponins-treated mice (Liang *et al.,* 2016).

The NMDA receptor plays an important role in the pathogenesis of clinical depression; NMDA receptor antagonists can treat this depression (Raab-Graham *et al.,* 2016). High concentration of NMDA induced decreases in norepinephrine, 5-HT, epinephrine, and dopamine in the brains of patients suffering from depression. Using an animal model for depression, Ginsenosides saponins showed protection against NMDA-induced depression *in vivo* and *in vi*tro. This indicates the potential application for the treatment of clinical depression (Zhang *et al.,* 2017).

Ginsenoside Rg3 (saponins) possesses well-confirmed immunoregulatory effects. Mice pretreatment with Rg3 reduced the immobility time in both the tail suspension test and the forced swimming test. Saponins attenuated the disturbed turnover of tryptophan and serotonin in the hippocampus, accompanied by decreased mRNA expression of pro-inflammatory cytokines and indoleamine-2,3-dioxygenase. Ginsenoside Rg3 saponins reduced lipopolysaccharide-induced elevation of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in plasma. This demonstrated that Rg3 was effective in ameliorating depressive-like behavior induced by immune activation (Kang *et al.,* 2017).

The transient potential receptor V1 (TRPV1) is involved in the etiology of depression. α-Phytosterol (Spinasterol) antagonizes the TRPV1 receptors and may produce antidepressant effects. α-Spinasterol may represent a new therapeutic approach toward the development of novel antidepressant therapy (Socała and Wlaź, 2016). Administration of phytosterols (β-sitosterol) produced an antidepressant-like effect similar to fluoxetine using FST and TST. It was confirmed that sterols affect monoamine levels in mice brains, and increased the brain levels of NE, and 5-HT (Zhao *et al.,* 2016).

Fucosterol sterol blocked the decrease in 5-HT, 5-HTIIA, and NA levels in mice brains generated by the stress of FST. The authors suggested that the antidepressant mechanism is mediated by increasing monoamines and reducing the rate of 5-HT metabolism. Therefore, fucosterol may be a useful antidepressant adjunct treatment for depression (Zhen *et al.,* 2015).

A significant increase in hippocampal brain-derived neurotrophic factor (BDNF) levels was found in the fucosterol (phytosterol) treated mice. These findings suggested that fucosterol may possess an antidepressant-like effect, which could be mediated by increasing central BDNF levels (Zhen *et al.,* 2015).

Muscle Relaxant of Calicotome Villosa Methanolic Extract

In the present study, methanolic extract of *Calicotome villosa* produced muscle relaxation. Using the Forced swim test and rotarod test, administration of extract of Prosopis cineraria leaves produces an antidepressant and muscle relaxant effect; the phytochemical screening revealed the presence of saponins, alkaloids, tannins, and phenolic compounds (George *et al.,* 2012).

Skeletal muscle relaxant activity of aqueous extract of *Nerium oleander* flowers in Albino rats may be due to the presence of alkaloids, tannins, and saponins in the plant extract. Authors suggested that skeletal muscle relaxation may be due to the agonistic effect on the GABA/benzodiazepine receptor complex (Tirumalasetti *et al.,* 2015).

Saponins detected in methanolic extract of *Calicotome villosa* may regulate calmodulin kinase channel (Ca 2+ / CaM / CaMK), and reduces the internal concentration of Ca2+ in nerve cells (Li *et al.,* 2010); also, piperine alkaloids may produce muscle relaxation by antagonizing sodium/calcium channel and GABA receptor agonistic effects (Mishra *et al.,* 2015). In another study, it was found that indole alkaloids have the ability to cross the blood-brain barrier (Zhang *et al.,* 2017) and potentially inhibit the Ca2+/calmodulin-dependent phosphodiesterase-1 and voltage-dependent Na+ channels (Hwang *et al.,* 2001).

Phenol is used for the treatment of acquired muscle spasticity; it can provide a temporary motor nerve blockade (D'Souza and Warner, 2022), leading to a muscle relaxation effect.

CNS Depression of Calicotome Villosa Methanolic Extract

In this study, methanolic extract of *Calicotome villosa* produced CNS depression by applying the Irwin test. This effect was not observed using the plus maze. Phenol produces transient CNS excitation (ATSDR, 2014), then profound CNS depression ensues rapidly (ATSDR, 2014; Dydek, 2014).

Different studies showed that saponins may produce either CNS stimulation or inhibitory action on CNS; it was found that saponins at high dose depress the CNS, while at low dose stimulate CNS. It was interpreted that saponins may produce their action on the CNS by a nonspecific action on the overall CNS (Hong *et al.,* 1974). This indicates that the methanolic extract of *Calicotome villosa* contains saponins in a concentration that is considered high; therefore, it produced a sedative effect in this study. Saponins might have a sedative action, with no effect on motor coordination, suggesting that it might not be acting through peripheral neuromuscular blockade (Capaso *et al.,* 1996). Another study confirmed that saponins had sedative activity; this effect might be via central mechanisms and not peripheral neuromuscular blockade (Perez *et al*., 1998).

It was reported that saponins have agonistic activities at the $GABA_A$ receptor complex (Shen *et al.,* 2020); this may be contributed to the CNS depressant effects in mice (Sultana *et al.,* 2018).

Alkaloids can cross the blood-brain barrier (Zhang *et al.,* 2017), and exerts a sedative effect (Jiang *et al.,* 2019). Indole alkaloids have been widely utilized in traditional treatments as a sedative (Munir *et al.,* 2020). The chemical structure of indole alkaloids has a similar structure as benzodiazepine drugs, therefore it may function via γ-aminobutyric acid receptors complex (Hamid *et al.,* 2017).

Beta-Carboline alkaloids are a large group of natural and synthetic indole alkaloids that are widely distributed in nature. These chemicals interact with benzodiazepine receptors and 5-hydroxy serotonin receptors and demonstrated a broad spectrum of pharmacological properties including sedative, anxiolytic, and hypnotic (Cao *et al.,* 2007).

Antipsychotics of Calicotome Villosa Methanolic Extract

Saponins may have antipsychotic and sedative activities (Chindo *et al.,* 2003; Shen *et al.,* 2020). It was found that saponins isolated from *Polygalae tenuifolia* were reported to possess antipsychotic effects (Chung *et al.,* 2002; Danjuma *et al.,* 2014).

It was reported that isoquinoline alkaloid has antipsychotic properties in rats (Alavijeh *et al.,* 2019). Alkaloids, act as a partial agonist of acetylcholine muscarinic receptor and showed an effect against schizophrenic symptoms (Hussain *et al.,* 2018). It directly stimulates the cholinergic activity and produces a potential stimulation of the CNS by binding to M2 muscarinic receptors (Hussain *et al.,* 2018). It was reported that alkaloids have AChE inhibiting properties in a dose-dependent manner (Dall'Acqua, 2013; Tundis *et al.,* 2009); therefore it ameliorates the cholinergic neurotransmission by reducing the ACh breakdown (Dall'Acqua, 2013).

The indole alkaloid alstonine acts as an atypical antipsychotic in behavioral models (Costa-Campos *et al.,* 2004). Another study showed that Indole alkaloid (Alstonine) is used to treat the mentally ill; it presents a clear antipsychotic profile in rodents. This could be through differential effects in distinct dopaminergic pathways (Linck *et al.,* 2011).

Gallic acid, as a constituent of tannins, reduced the dopamine levels, AChE activity, and inflammatory surge (serum TNF-α), and increased the levels of GABA in mice. These studies indicate that tannins could protect and ameliorate psychotic symptoms and biochemical changes in mice (Yadav *et al.,* 2017; Otimenyin and Ior, 2021).

CONCLUSION

Preliminary phytochemical screening of *Calycatome villosa* methanolic extract revealed the presence of alkaloids, saponins, carbohydrates, phytosterols, phenols, and tannins. The pharmacological screening of the extract showed antidepressant, antipsychotic, CNS depression, and Muscle relaxant effects. The extract showed CNS depression using the Irwin test, while the spontaneous motor activity was not changed using EPM. *Calycatome villosa* methanolic extract with the dose used did not show an antianxiety-like effect using EPM.

REFERENCES

- Abdelkarim, G., Alami, I., Malika, A., Boukhatem, N., Lechkar, M. & Hamal, A. (2016). Contribution to Botanical, Phyto-ecological and Phytochemical Studies of Calicotome villosa (Poiret) Link subsp. intermedia (C. Presl): A Phylogenetic Approach from Moroccan Species. *International Journal of Green and Herbal Chemistry*, 5, 2278-3229.

- Aburawi, S. M. (1999). Study of neuro chemical mechanisms involved in tolerance and physical dependence to traizolam in experimental animals, Ph.D. Thesis, Cairo University.
- Adell, A., Biggs, T. A., & Myers, R. D. (1996). Action of harman (1-methyl-β-carboline) on the brain: Body temperature and in vivo efflux of 5-HT from hippocampus of the rat. *Neuropharmacology*, 35(8), 1101-1107.
- Agency for Toxic Substances and Disease Registry (ATSDR). Medical Management Guidelines for Phenol. Page last reviewed October 21, 2014.
- Akomolafe, S. F., Akinyemi, A. J., Ogunsuyi, O. B., Oyeleye, S. I., Oboh, G., Adeoyo, O. O., & Allismith, Y. R. (2017). Effect of caffeine, caffeic acid and their various combinations on enzymes of cholinergic, monoaminergic and purinergic systems critical to neurodegeneration in rat brain—in vitro. *NeuroToxicology*, 62, 6-13.
- Alavijeh, M. M., Vaezi, G., Khaksari, M., & Hojati, V. (2019). Berberine hydrochloride attenuates voluntary methamphetamine consumption and anxiety-like behaviors via modulation of oxytocin receptors in methamphetamine addicted rats. *Physiology & behavior*, 206, 157-165.
- Albert, P. R. (2019). Targeting Homer1a for rapid antidepressant effects. *Neuron*, *104*(2), 182-183.
- Ali, B. H., Bashir, A. K., Tanira, M. O. M., Medvedev, A. E., Jarrett, N., Sandler, M., & Glover, V. (1998). Effect of extract of Rhazya stricta, a traditional medicinal plant, on rat brain tribulin. *Pharmacology Biochemistry and Behavior*, 59(3), 671-675.
- Aristidis M. Tsatsakis, Aristidis S. Veskoukis, Christina Tsitsimpikou, Ioannis Tsatsakis, Ramin Rezaee (2021) Safety profile of plants and phytoconstituents used in traditional medicine worldwide. In: Toxicological Risk Assessment and Multi-System Health Impacts from Exposure. Editors: Aristidis M. Tsatsakis. Chapter 37, Pages 435-447. Academic Press.
- Arnason, J. T., Catling, P. M., Small, E., Dang, P. T., & Lambert, J. D. H. (Eds.) (2005). Biodiversity & health: focusing research to policy. Proceedings of the international symposium, held in Ottawa, Canada, October 25-28, 2003, NRC Research Press. (Book)
- Bai, J., Dong, X., He, S., & Bao, M. (2017). Monocular deprivation of Fourier phase information boosts the deprived eye's dominance during interocular competition but not interocular phase combination. *Neuroscience*, 352, 122-130.
- Baird-Lambert, J., Davis, P. A., & Taylor, K. M. (1982). Methylaplysinopsin: a natural product of marine origin with effects on serotonergic neurotransmission. *Clinical and Experimental Pharmacology & Physiology*, 9(2), 203-212.
- Bhat, S. K., Ashwin, D., & Sarpangala, M. (2017). Contamination and adulteration in arecanut (areca catechu L.) and its chewing forms: the less focused subject by health researchers. *IOSR J. Environ. Sci. Toxicol. Food Tech*, 11(1), 07-12.
- Cao, R., Peng, W., Wang, Z., & Xu, A. (2007). β-Carboline alkaloids: biochemical and pharmacological functions. *Current medicinal chemistry*, 14(4), 479-500.
- Capasso, A., De Feo, V., De Simone, F., & Sorrentino, L. (1996). Pharmacological effects of aqueous extract from Valeriana adscendens. *Phytotherapy Research*, 10(4), 309-312.
- Cappelletti, S., Daria, P., Sani, G., & Aromatario, M. (2015). Caffeine: Cognitive and Physical Performance Enhancer or Psychoactive Drug? *Current Neuropharmacology*, 13(1), 71-88.
- Carlini, E. A. (2003). Plants and the central nervous system. *Pharmacology Biochemistry and Behavior*, 75(3), 501-512.
- Chikhi, I., Allali, H., Bechlaghem, K., Fekih, N., Muselli, A., Djabou, N., Dib, M., Tabti, B., Halla, N. & Costa, j., (2014). Assessment of in vitro antimicrobial potency and free radical scavenging capacity of the essential oil and ethanol extract of Calycotome villosa subsp. intermedia growing in Algeria. *Asian Pacific Journal of Tropical Disease*, 4(5), 356-362.
- Chung, I. W., Moore, N. A., Oh, W. K., O'Neill, M. F., Ahn, J. S., Park, J. B., ... & Kim, Y. S. (2002). Behavioural pharmacology of polygalasaponins indicates potential antipsychotic efficacy. *Pharmacology Biochemistry and Behavior*, 71(1-2), 191-195.
- Cleveland Heartlab (2017) Caffeine, the Heart, and Inflammation. March 13, 2017 DIET.
- Costa-Campos, L., Dassoler, S.C., Rigo, A.P., Iwu, M., & Elisabetsky, E. (2004) Anxiolytic properties of the antipsychotic alkaloid alstonine. *Pharmacol Biochem Behav.*, 77(3), 481-9.
- Crema, L. M., Pettenuzzo, L. F., Schlabitz, M., Diehl, L., Hoppe, J., Mestriner, R., ... & Vendite, D. (2013). The effect of unpredictable chronic mild stress on depressive-like behavior and on hippocampal A1 and striatal A2A adenosine receptors. *Physiology & behavior*, 109, 1-7.
- Cryan, J.F., Page, M.E., Luki, I. (2002). Noradrenergic lesions differentially alter the antidepressant like effects of reboxetine in a modified forced swim test. *Eur. J. Pharmacol.*, 436, 179-205.
- Cunha R. A., Johansson B., van der Ploeg I., Sebastiao A. M., Ribeiro J. A., & Fredholm B. B. (1994) Evidence for functionally important adenosine A2a receptors in the rat hippocampus. *Brain Res.*, 649, 208– 216.
- Dall'Acqua, S. (2013). Plant-derived acetylcholinesterase inhibitory alkaloids for the treatment of Alzheimer's disease. *Botanics: Targets and Therapy*, 3, 19-28.
- Daly, J. W. (1993). Mechanism of action of caffeine. *Caffeine, coffee, and health*, 97-150.

- Daly, J. W., Shi, D., Nikodijević, O., & Jacobson, K. A. (2020). The role of adenosine receptors in the central action of caffeine. In *Caffeine and Behavior* (pp. 1-16). CRC Press.
- Danjuma, N. M., Chindo, B. A., Abdu-Aguye, I., Anuka, J. A., & Hussaini, I. M. (2014). Psychopharmacological properties of saponins from Randia nilotica stem bark. *Pharmaceutical Biology*, 52(1), 1-7.
- Dhingra, D., & Valecha, R. (2014). Punarnavine, an alkaloid isolated from ethanolic extract of Boerhaavia diffusa Linn. reverses depression-like behaviour in mice subjected to chronic unpredictable mild stress. *Indian journal of experimental biology*, 52(8)799-807
- D'Souza, R. S., & Warner, N. S. (2022). Phenol Nerve Block. [Updated 2022 Apr 30]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK525978/
- Dydek, S. T. (2014) Shale Oil Toxicity. In: Encyclopedia of Toxicology (Third Edition). Editor(s): Philip Wexler. Pages 248-251. *Academic Press.*
- Fajemiroye, J. O., da Silva, D. M., de Oliveira, D. R., & Costa, E. A. (2016). Treatment of anxiety and depression: medicinal plants in retrospect. *Fundamental & clinical pharmacology*, *30*(3), 198-215.
- Farouk, L., Laroubi, A., Aboufatima, R., Benharref, A. & Chait, A. (2008). Evaluation of the analgesic effect of alkaloid extract of Peganum harmala L.: possible mechanisms involved. *Journal of Ethnopharmacology.,* 115(3), 449–454.
- Farzin, D., & Mansouri, N. (2006) Antidepressant-like effect of harmane and other β-carbolines in the mouse forced swim test. *European Neuropsychopharmacology,* 16, 324-328
- Farzin, D., & Mansouri, N. (2006). Antidepressant-like effect of harmane and other β-carbolines in the mouse forced swim test. *European Neuropsychopharmacology.*, 16, 324-328
- Fortunato, J. J., Réus, G. Z., Kirsch, T. R., Stinagari, R. B., Stretz L., Kapczinski, F., Pinto, J. P., Hallak, J. E., Zuardi, A. W., Crippa, J. A., & Quevedo, J. (2009). Acute harmine administration induces antidepressive-like effects and increases BDNF levels in the rat hippocampus. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 33(8), 1425–1430.
- Fortunato, J. J., Réus, G. Z., Kirsch, T. R., Stringari, R. B., Fries, G. R., Kapczinski, F., ... & Quevedo, J. (2010). Effects of β-carboline harmine on behavioral and physiological parameters observed in the chronic mild stress model: Further evidence of antidepressant properties. *Brain research bulletin*, 81(4-5), 491-496.
- Gałecki, P., Mossakowska-Wójcik, J., & Talarowska, M. (2018). The anti-inflammatory mechanism of antidepressants–SSRIs, SNRIs. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 80, 291-294.
- García-Ríos, R. I., Mora-Pérez, A., Ramos-Molina, A. R., & Soria-Fregozo, C. (2020). Neuropharmacology of Secondary Metabolites from Plants with Anxiolytic and Antidepressant Properties. In Behavioral Pharmacology-From Basic to Clinical Research. London, UK: IntechOpen.
- George, M., Joseph, L., & Sharma, A. (2012). Antidepressant and skeletal muscle relaxant effects of the aqueous extract of the Prosopis cineraria. *Brazilian journal of pharmaceutical sciences*, 48, 577-581.
- Glennon, R. A., Dukat, M., Grella, B., Hong, S. S., Costantino, L., Teitler, M., ... & Mattson, M. V. (2000). Binding of β-carbolines and related agents at serotonin (5-HT2 and 5-HT1A), dopamine (D2) and benzodiazepine receptors. *Drug and alcohol dependence*, 60(2), 121-132.
- Guan, L.P. & Liu, B.Y. (2016). Antidepressant-like effects and mechanisms of flavonoids and related analogues, *European Journal of Medicinal Chemistry*, 121, 47–57.
- Hamid, H. A., Ramli, A. N., & Yusoff, M. M. (2017). Indole alkaloids from plants as potential leads for antidepressant drugs: A mini review. *Frontiers in pharmacology*, 8, 96.
- Hanapi, N. A., Chear, N. J. Y., Azizi, J., & Yusof, S. R. (2021). Kratom Alkaloids: Interactions with Enzymes, Receptors, and Cellular Barriers. *Frontiers in Pharmacology*, 12.
- Harvey, A. L., Clark, R. L., Mackay, S. P., & Johnston, B. F. (2010). Current strategies for drug discovery through natural products. *Expert opinion on drug discovery*, 5(6), 559-568.
- Hedner, E., Sjögren, M., Frändberg, P.-A., Johansson, T., Göransson, U., Dahlström, M., Jonsson, P., Nyberg, F. & Bohlin, Lars. (2006). Brominated cyclodipeptides from the marine spongegeodiabarrettias selective 5-HT ligands. *Journal of Natural Products*, 69(10), 1421–1424
- Herraiz, T., & Guillén, H. (2011). Inhibition of the bioactivation of the neurotoxin MPTP by antioxidants, redox agents and monoamine oxidase inhibitors. *Food and Chemical Toxicology*, 49(8), 1773–1781.
- Hodgson, R. A., Bertorelli, R., Varty, G. B., Lachowicz, J. E., Forlani, A., Fredduzzi, S., ... & Hunter, J. C. (2009). Characterization of the potent and highly selective A2A receptor antagonists preladenant and SCH 412348 [7-[2-[4-2, 4-difluorophenyl]-1-piperazinyl] ethyl]-2-(2-furanyl)-7H-pyrazolo [4, 3-e][1, 2, 4] triazolo [1, 5-c] pyrimidin-5-amine] in rodent models of movement disorders and depression. *Journal of Pharmacology and Experimental Therapeutics*, 330(1), 294-303.
- Hong, S. A., Park, C. W., Kim, J. H., Chang, H. K., Hong, S. K., & Kim, M. S. (1974). The effect of ginseng saponins on animal behavior. This article is the English translation of the original report written in Korean. *Korean J. of Pharm.*, 10(1), 1 – 11.

- Howren, M. B., Lamkin, D. M., & Suls, J. (2009). Associations of depression with C-reactive protein, IL-1, and IL-6: a meta-analysis. *Psychosomatic medicine*, 71(2), 171-186.
- Hussain, G., Rasul, A., Anwar, H., Aziz, N., Razzaq, A., Wei, W., ... & Li, X. (2018). Role of plant derived alkaloids and their mechanism in neurodegenerative disorders. *International journal of biological sciences*, 14(3), 341.
- Hwang, J. H., Kim, K. J., Ryu, S. J., & Lee, B. Y. (2016). Caffeine prevents LPS-induced inflammatory responses in RAW264.7 cells and zebrafish. *Chem Biol Interact.*, 248, 1-7.
- Hwang, K. H., Han, Y. N., & Han, B. H. (2001). Inhibition of calmodulin-dependent calcium-ATPase and phosphodiesterase by various cyclopeptides and peptide alkaloids from the Zizyphus species. *Archives of Pharmacal Research*, 24(3), 202-206.
- Idayu, N. F., Hidayat, M. T., Moklas, M. A. M., Sharida, F., Raudzah, A. N., Shamima, A. R., & Apryani, E. (2011). Antidepressant-like effect of mitragynine isolated from Mitragyna speciosa Korth in mice model of depression. *Phytomedicine*, 18(5), 402-407.
- János, B. (2005). Bioactive Microbial Metabolites. *Review. J. Antibiot.*, 58(1), 1–26.
- Jawna-Zboińska, K., Blecharz-Klin, K., Joniec-Maciejak, I., Wawer, A., Pyrzanowska, J., Piechal, A., & Widy-Tyszkiewicz, E. (2016). Passiflora incarnata L. improves spatial memory, reduces stress, and affects neurotransmission in rats. *Phytotherapy Research*, 30(5), 781-789.
- Jiang, B., Xiong, Z., Yang, J., Wang, W., Wang, Y., Hu, Z. L., wang, F., & Chen, J. G. (2012). Antidepressant-like effects of ginsenoside Rg1 are due to activation of the BDNF signalling pathway and neurogenesis in the hippocampus. *British journal of pharmacology*, 166(6), 1872-1887.
- Jiang, M. L., Zhang, Z. X., Li, Y. Z., Wang, X. H., Yan, W., & Gong, G. Q. (2015). Antidepressant-like effect of evodiamine on chronic unpredictable mild stress rats. *Neuroscience Letters*, 588, 154-158.
- Jiang, Y. P., Chen, Z. Y., Mo, F., Liu, M., Huang, Q., & Liu, S. (2019). Pharmacological mechanism of traditional sedative effect of alkaloids in Nelumbinis Plumula based on network pharmacology. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, 44(19), 4225-4233.
- Kale, P. P., Addepalli, V., Bafna, P. A., & Prabhavalkar, K. S. (2010). Caffeine-induced Augmentation of Antidepressant Therapy. *J. Exp. Clin. Med.*, 2(6), 282–286.
- Kang, A., Xie, T., Zhu, D., Shan, J., Di, L., & Zheng, X. (2017). Suppressive effect of ginsenoside Rg3 against lipopolysaccharide-induced depression-like behavior and neuroinflammation in mice. *Journal of agricultural and food chemistry*, 65(32), 6861-6869.
- Kaster, M. P., Machado, N. J., Silva, H. B., Nunes, A., Ardais, A. P., Santana, M., ... & Cunha, R. A. (2015). Caffeine acts through neuronal adenosine A2A receptors to prevent mood and memory dysfunction triggered by chronic stress. *Proceedings of the National Academy of Sciences*, 112(25), 7833-7838.
- Kohler, O., Krogh, J., Mors, O., & Eriksen Benros, M. (2016). Inflammation in depression and the potential for anti-inflammatory treatment. *Current neuropharmacology*, 14(7), 732-742.
- Köroğlu, Ö. A., MacFarlane, P. M., Balan, K. V., Zenebe, W. J., Jafri, A., Martin, R. J., & Kc, P. (2014). Anti-inflammatory effect of caffeine is associated with improved lung function after lipopolysaccharide-induced amnionitis. *Neonatology*, 106(3), 235-240.
- Kumar, S., & Sharma, A. (2005). Anti-anxiety Activity Studies on Homoeopathic Formulations ofTurnera aphrodisiacaWard. *Evidence-Based Complementary and Alternative Medicine*, 2(1), 117–119.
- Kumarnsit, E., Vongvatcharanon, U., Keawpradub, N., & Intasaro, P. (2007). Fos-like immunoreactivity in rat dorsal raphe nuclei induced by alkaloid extract of Mitragyna speciosa. *Neuroscience Letters*, 416(2), 128–132.
- Küpeli Akkol, E., Tatlı Çankaya, I., Şeker Karatoprak, G., Carpar, E., Sobarzo-Sánchez, E., & Capasso, R. (2021). Natural compounds as medical strategies in the prevention and treatment of psychiatric disorders seen in neurological diseases. *Frontiers in Pharmacology*, 12, 1-27.
- Lee, B. B., Shim, I. S., Lee, H. J., & Hahm, D. H. (2012). Effect of ginsenoside Re on depression-and anxiety-like behaviors and cognition memory deficit induced by repeated immobilization in rats. *Journal of Microbiology and Biotechnology*, 22(5), 708-720.
- Leff-Gelman, P., Mancilla-Herrera, I., Flores-Ramos, M., Cruz-Fuentes, C., Reyes-Grajeda, J. P., García-Cuétara, M. D. P., ... & Pulido-Ascencio, D. E. (2016). The immune system and the role of inflammation in perinatal depression. *Neuroscience bulletin*, 32(4), 398-420.
- Li, Q., Guan, Y., Ma, H. J., Guo, Z., He, R. R., & Zhang, Y. (2010). Influences of Panax notoginsenosid on spontaneous contraction of small intestine smooth muscle of rabbits in vitro. *Zhongguo Ying Yong Sheng li xue za zhi= Zhongguo Yingyong Shenglixue Zazhi= Chinese Journal of Applied Physiology*, 26(1), 117-120.
- Liang, Y., Yang, X., Zhang, X., Duan, H., Jin, M., Sun, Y., ... & Qiao, W. (2016). Antidepressant-like effect of the saponins part of ethanol extract from SHF. *Journal of ethnopharmacology*, 191, 307-314.
- Lin, Y., Zhao, W-R., Shi, W-T, Zhang, J., Zhang, K-Y., Ding, Q., Chen, X-L., Tang, J-Y., Zhou, Z-Y. (2020). Pharmacological Activity, Pharmacokinetics, and Toxicity of Timosaponin AIII, a Natural Product Isolated from Anemarrhena asphodeloides Bunge: A review. *Frontiers in Pharmacology*, 11,764.
- Linck, V. M., Herrmann, A. P., Piato, Â. L., Detanico, B. C., Figueiró, M., Flório, J., ... & Elisabetsky, E. (2011).

Alstonine as an antipsychotic: Effects on brain amines and metabolic changes. *Evidence-Based Complementary and Alternative Medicine*, *2011*, 418597. doi: 10.1093/ecam/nep002.

- Liu, J., Qiao, W., Yang, Y., Ren, L., Sun, Y., & Wang, S. (2012). Antidepressant-like effect of the ethanolic extract from Suanzaorenhehuan Formula in mice models of depression. *Journal of Ethnopharmacology*, 141(1), 257-264.
- Liu, M. Y., Zhang, L. J., & Liu, M. C. (2016). Effect of ginsenoside pre-treatment on 5-hydroxytryptamine system in SD rats with myocardial infarction and depression. *Zhonghua nei ke za zhi*, 55(9), 700-704.
- López-Cruz, L., Salamone, J. D., & Correa, M. (2018). Caffeine and selective adenosine receptor antagonists as new therapeutic tools for the motivational symptoms of depression. *Frontiers in pharmacology*, 9, 526.
- Lundstrom, K., Pham, H. T., & Dinh, L. D. (2017). Interaction of plant extracts with central nervous system receptors. *Medicines*, 4(1), 12.
- Marek, K. L., & Roth, R. H. (1980). Ergot alkaloids: interaction with presynaptic dopamine receptors in the neostriatum and olfactory tubercles. *European journal of pharmacology*, 62(2-3), 137-146.
- McCarter, T. (2008). Depression overview. *American Health & Drug Benefits*, 1(3), 44–51.
- Mikkilineni, S., Cantuti-Castelvetri, I., Cahill, C. M., Balliedier, A., Greig, N. H., & Rogers, J. T. (2012). The anticholinesterase phenserine and its enantiomer posiphen as 5′ untranslated-region-directed translation blockers of the Parkinson's alpha synuclein expression. *Parkinson's Disease*, 2012,5-8
- Mishra, A., Punia, J. K., Bladen, C., Zamponi, G. W., & Goel, R. K. (2015). Anticonvulsant mechanisms of piperine, a piperidine alkaloid. *Channels*, 9(5), 317-323.
- More, S. V., Kumar, H., Kim, I. S., Song, S. Y., & Choi, D. K. (2013). Cellular and molecular mediators of neuroinflammation in the pathogenesis of Parkinson's disease. *Mediators of inflammation*, 2013.
- Moser, V. C. (2010). Functional Assays for Neurotoxicity Testing. *Toxicologic Pathology*, 39, 36-45.
- Müller, N., Schwarz, M. J., Dehning, S., Douhe, A., Cerovecki, A., Goldstein-Müller, B., ... & Riedel, M. (2006). The cyclooxygenase-2 inhibitor celecoxib has therapeutic effects in major depression: results of a double-blind, randomized, placebo controlled, add-on pilot study to reboxetine. *Molecular psychiatry*, 11(7), 680-684.
- Munir, S., Shahid, A., Aslam, B., Ashfaq, U. A., Akash, M. S. H., Ali, M. A., ... & Khurshid, M. (2020). The therapeutic prospects of naturally occurring and synthetic indole alkaloids for depression and anxiety disorders. *Evidence-Based Complementary and Alternative Medicine*, *2020*.
- Nasehi, M., Piri, M., Nouri, M., Farzin, D., Nayer-Nouri, T. & Zarrindast, M. R. (2010). Involvement of dopamine D1/D2 receptors on harmane-induced amnesia in the step-down passive avoidance test. *European Journal of Pharmacology*, 634(1–3), 77–83.
- Otimenyin, S. O., & Ior, L. D. (2021). Medicinal Plants Used in the Management of Psychosis. In Complementary Therapies. IntechOpen.
- Perera, P., Kanjanapothy, D., Sandberg, F., & Verpoorte, R. (1985). Muscle relaxant activity and hypotensive activity of some Tabernaemontana alkaloids. *Journal of ethnopharmacology*, 13(2), 165-173.
- Perez G, R. M., Perez L, J. A., Garcia D, L. M., & Sossa M, H. (1998). Neuropharmacological activity of Solanum nigrum fruit. *Journal of ethnopharmacology*, 62(1), 43-48.
- Pisetsky, D. S. (2014). The translocation of nuclear molecules during inflammation and cell death. *Antioxid Redox Signal. 1*, 20(7), 1117-25.
- Qi, H., Huang, Y., Yang, Y., Dou, G., Wan, F., Zhang, W., ... & Li, L. (2016). Anti-platelet activity of panaxatriol saponins is mediated by suppression of intracellular calcium mobilization and ERK2/p38 activation. *BMC Complementary and Alternative Medicine*, 16(1), 1-8.
- Raab-Graham, K. F., Workman, E. R., Namjoshi, S., & Niere, F. (2016). Pushing the threshold: How NMDAR antagonists induce homeostasis through protein synthesis to remedy depression. *Brain research*, 1647, 94-104.
- Rodgers, R. J. (1997). Animal models of anxiety: where next? *Behav. Pharmacol.*, 8(6-7), 477-496.
- Rojeck, L. B., Kalodera, Z., & Samarzija, I. (2004). The antidepressant activity of Hypericum perforatum L. measured by two experimental methods on mice. *Acta Pharma.*, 54, 157-162.
- Satti, A. A., & Edriss, A. E. (2013). Phytochemical analyses of three sudanese plants for their constituents of bioactive compounds. *Int J Sci Nat*, 4, 169-73.
- Shen, C. Y., Ma, P. Y., Zhu, J. J., Jiang, J. G., Liu, L., Yi, Y. K., ... & Liu, Q. (2020). Saponin extracts from Gynostemma pentaphyllum (Thunb.) Makino display sedative-hypnotic and anxiolytic effects. *Industrial Crops and Products*, 157, 112893.
- Shushtari, N., & Froushani, S. M. A. (2017). Caffeine augments the instruction of anti-inflammatory macrophages by the conditioned medium of mesenchymal stem cells. *Cell Journal (Yakhteh)*, *19*(3), 415-424.
- Sichardt, K., Vissiennon, Z., Koetter, U., Brattström, A., & Nieber, K. (2007). Modulation of postsynaptic potentials in rat cortical neurons by valerian extracts macerated with different alcohols: involvement of adenosine A1- and GABAA-receptors. *Phytotherapy Research*, 21(10), 932-937.
- Smith, K. L., Ford, G. K., Jessop, D. S., & Finn, D. P. (2013). Behavioural, neurochemical and neuroendocrine effects of the endogenous β-carboline harmane in fear-conditioned rats. *Journal of Psychopharmacology*, 27(2), 162-170.

- Smith, R. S. (1991). The macrophage theory of depression. *Medical hypotheses*, 35(4), 298-306.
- Socała, K., & Wlaź, P. (2016). Evaluation of the antidepressant-and anxiolytic-like activity of α-spinasterol, a plant derivative with TRPV1 antagonistic effects, in mice. *Behavioural Brain Research*, 303, 19-25
- Splettstoesser, F., Bonnet, U., Wiemann, M., Bingmann, D. & Büsselberg, D. (2005). Modulation of voltage-gated channel currents by harmaline and harmane. *British Journal of Pharmacology*, 144(1), 52–58.
- Stockwell, J., Jakova, E., & Cayabyab, F. S. (2017). Adenosine A1 and A2A receptors in the brain: current research and their role in neurodegeneration. *Molecules*, 22(4), 676.
- Su, P., Wang, L., Du, S. J., Xin, W. F., & Zhang, W. S. (2014). Advance in studies of Panax notoginseng saponins on pharmacological mechanism of nervous system disease. *Zhongguo Zhong yao za zhi= Zhongguo zhongyao zazhi= China journal of Chinese materia medica*, 39(23), 4516-4521.
- Sultana, T., Mannan, M., & Ahmed, T. (2018). Evaluation of central nervous system (CNS) depressant activity of methanolic extract of Commelina diffusa Burm. in mice. *Clinical Phytoscience*, 4(1), 1-7.
- Taylor, C., Fricker, A. D., Devi, L. A., & Gomes, I. (2005). Mechanisms of action of antidepressants: from neurotransmitter systems to signaling pathways. *Cell Signal.*, 17(5), 549-57
- Temitope, A. O., & Santwana, P. (2021). Herbal remedies, toxicity, and regulations. In: Preparation of Phytopharmaceuticals for the Management of Disorders. Editors: Chukwuebuka Egbuna and Abhay Prakash Mishra and Megh R. Goyal. Chapter 6, Page 89-127. *Academic Press.*
- Tirumalasetti, J., Patel, M., Shaikh, U., Harini, K., & Shankar, J. (2015). Evaluation of skeletal muscle relaxant activity of aqueous extract of Nerium oleander flowers in Albino rats. *Indian journal of pharmacology*, 47(4), 409-413
- Tiwari, P., Kaur, M., & Kaur, H. (2011). Phytochemical screening and Extraction: A Review. *Internationale Pharmaceutica Sciencia*, 1, 98- 106.
- Tsai, S. J., Chao, C. Y., & Yin, M. C. (2011). Preventive and therapeutic effects of caffeic acid against inflammatory injury in striatum of MPTP-treated mice. *European Journal of Pharmacology*, 670(2-3), 441-447.
- Tundis, R., Menichini, F., Conforti, F., Loizzo, M. R., Bonesi, M., Statti, G., & Menichini, F. (2009). A potential role of alkaloid extracts from Salsola species (Chenopodiaceae) in the treatment of Alzheimer's disease. *Journal of enzyme inhibition and medicinal chemistry*, 24(3), 818-824.
- Tyring, S., Gottlieb, A., Papp, K., Gordon, K., Leonardi, C., Wang, A., ... & Krishnan, R. (2006). Etanercept and clinical outcomes, fatigue, and depression in psoriasis: double-blind placebo-controlled randomised phase III trial. *The Lancet*, 367(9504), 29-35.
- Ubedulla, S., Jayasree, T., Chandra Sekhar, N., & Prakash, M. (2014). Evaluation of skeletal muscle relaxant activity of aqueous extract of Nerium oleander leaves in albino rats. *Int J Biol Pharm Res*, 5, 191-5.
- van Calker, D., Biber, K., Domschke, K., & Serchov, T. (2019). The role of adenosine receptors in mood and anxiety disorders. *Journal of neurochemistry*, 151(1), 11-27.
- Vikas, G., & Payal, M. (2010). Phytochemical and pharmacological potential of Nerium oleander: a review. *International Journal of Pharmaceutical Sciences and Research (IJPSR)*, 1(3), 21-27.
- Vinader-Caerols, C., Martos, A. J., Monleón, S., Arenas, M. C., & Parra, A. (2006). Acute effects of maprotiline on learning, anxiety, activity, and analgesia in male and female mice. *Acta neurobiologiae experimentalis*, 66(1), 23-31.
- Wang, H., Zhang, K., Zhao, L., Tang, J., Gao, L., & Wei, Z. (2014). Anti-inflammatory effects of vinpocetine on the functional expression of nuclear factor-kappa B and tumor necrosis factor-alpha in a rat model of cerebral ischemia–reperfusion injury. *Neuroscience letters*, 566, 247-251.
- WHO, Depression. 13 September 2021. https://www.who.int/news-room/fact-sheets/detail/depression
- Woo, T. U. W. (2013). Neurobiology of schizophrenia onset. *The neurobiology of childhood*, 267-295.
- Wu, H. F., Zhu, C. H., & Guo, J. Y. (2012). Effect of ginsenoside Rg1 on behaviors and hippocampal amino acids in depressive-like rats. *Zhongguo Zhong yao za zhi= Zhongguo Zhongyao Zazhi= China Journal of Chinese Materia Medica*, 37(20), 3117-3121.
- Xiang, H., Liu, Y., Zhang, B., Huang, J., Li, Y., Yang, B., ... & Zhang, H. (2011). The antidepressant effects and mechanism of action of total saponins from the caudexes and leaves of Panax notoginseng in animal models of depression. *Phytomedicine*, 18(8-9), 731-738.
- Xie, W., Meng, X., Zhai, Y., Zhou, P., Ye, T., Wang, Z., ... & Sun, X. (2018). Panax notoginseng saponins: a review of its mechanisms of antidepressant or anxiolytic effects and network analysis on phytochemistry and pharmacology. *Molecules*, 23(4), 940.
- Yadav, M., Jindal, D. K., Dhingra, M. S., Kumar, A., Parle, M., & Dhingra, S. (2018). Protective effect of gallic acid in experimental model of ketamine-induced psychosis: possible behaviour, biochemical, neurochemical and cellular alterations. *Inflammopharmacology*, 26(2), 413-424.
- Yamada, K., Kobayashi, M., Mori, A., Jenner, P., & Kanda, T. (2013). Antidepressant-like activity of the adenosine A2A receptor antagonist, istradefylline (KW-6002), in the forced swim test and the tail suspension test in rodents. *Pharmacology Biochemistry and Behavior*, 114, 23-30.
- You, Z., Yao, Q., Shen, J., Gu, Z., Xu, H., Wu, Z., Chen, C., & Li, L. (2017). Antidepressant-like effects of

ginsenoside Rg3 in mice via activation of the hippocampal BDNF signaling cascade. *Journal of Natural Medicines*, 71(2), 367-379.

- Yu, A. M., Idle, J. R., Krausz, K. W., Küpfer, A., & Gonzalez, F. J. (2003). Contribution of Individual Cytochrome P450 Isozymes to theO-Demethylation of the Psychotropic β-Carboline Alkaloids Harmaline and Harmine. *Journal of Pharmacology and Experimental Therapeutics*, 305(1), 315-322.
- Zhang, H., Li, Z., Zhou, Z., Yang, H., Zhong, Z., & Lou, C. (2016). Antidepressant-like effects of ginsenosides: a comparison of ginsenoside Rb3 and its four deglycosylated derivatives, Rg3, Rh2, compound K, and 20 (S)-protopanaxadiol in mice models of despair. *Pharmacology Biochemistry and Behavior*, 140, 17-26.
- Zhang, H., Zhou, Z., Chen, Z., Zhong, Z., & Li, Z. (2017). Ginsenoside Rg3 exerts anti-depressive effect on an NMDA-treated cell model and a chronic mild stress animal model. *Journal of Pharmacological Sciences*, 134(1), 45-54.
- Zhang, Y. N., Yang, Y. F., Xu, W., & Yang, X. W. (2017). The Blood-Brain Barrier Permeability of Six Indole Alkaloids from Uncariae Ramulus Cum Uncis in the MDCK-pHaMDR Cell Monolayer Model. *Molecules (Basel, Switzerland)*, 22(11), 1944.
- Zhao, D., Zheng, L., Qi, L., Wang, S., Guan, L., Xia, Y., & Cai, J. (2016). Structural features and potent antidepressant effects of total sterols and β-sitosterol extracted from Sargassum horneri. *Marine drugs*, 14(7), 123.
- Zhen, X. H., Quan, Y. C., Jiang, H. Y., Wen, Z. S., Qu, Y. L., & Guan, L. P. (2015). Fucosterol, a sterol extracted from Sargassum fusiforme, shows antidepressant and anticonvulsant effects. *European Journal of Pharmacology*, 768, 131-138.