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PHYTOCHEMICAL SCREENING AND ANTI-ANXIETY ACTIVITY OF METHANOLIC AND CHLOROFORM EXTRACTS OF *LANNEA COROMANDELICA* LEAVES IN WISTER ALBINO MICE MODEL

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Abstract:

Aim: *This study aimed to evaluate the anti-anxiety activity of the Methanolic extract, and chloroform extract of Lannea coromandelica leaves in mice using an animal model.* ***Methods****: Psychopharmacological studies present antioxidants as a potential new strategy for the treatment of anxiety. The present paper deals with a preliminary phytochemical evaluation of the leaf Lannea coromandelica to establish anti-anxiety activity.* ***Result:*** *The study includes the preparation of different extracts and their phytochemical analysis and biological activity. Phytochemical studies revealed the presence of saponins, tannins, phenolics, terpenoids, and steroids. In the present investigation, Methanolic extract at 500 (µg/ml) showed 44.11% Scavenging effect & Chloroform extract at 500 (µg/ml) showed 51.47 %. These extracts showed moderate radical scavenging activity in the DPPH assay. This may be attributable to the high total phenolic and total flavonoid contents of the extracts. Elevated plus maze test was used to assess the psychomotor performance and emotional aspects of mice, treatment with Methanolic extract, and chloroform extract revealed anxiolytic activity, since the number of open arm entries and time spent in open arm parameters are the most delegated guide for anxiolytic activity* ***Discussion:*** *In our study, we demonstrated significant anti-anxiety activity with 500mg of both extracts compared to control group but has less activity when compared to that of diazepam but both were statistically significant. The average of basal activity scores in the control group after 30 and 60 minutes of administration of Methanolic, chloroform extracts of Lannea coromandelica leaves 500 mg/kg p.o. significantly reduced locomotor activity.* ***Conclusion****: It may be due to the CNS depressant property of the drug. The time spent by the animal in the central platform seems to be related to decision-making or risk assessment and the total arm entries is a measure reflecting changes in anxiety.*

Keywords: *Lannea coromandelica, locomotor activity, anti-anxiety, DPPH assay*

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INTRODUCTION:

"Currently, herbal medicines are widely used as a natural alternative for healthcare due to their safety and reliable health benefits. Traditional herbal medicines have gained increasing attention in recent decades because of their remarkable pharmacological activities, cost-effectiveness, and minimal side effects in healthcare management."[1]

Drugs that affect the central nervous system (CNS) are commonly used as medicinal agents [2]. CNS depressants like barbiturates, benzodiazepines, and ethanol interact with the postsynaptic gamma-aminobutyric acid receptor (GABAA receptor) to produce their effects [3]. However, the use of barbiturates as a CNS depressant is limited due to their narrow margin of safety; just 10 times their therapeutic dose can be lethal [4]. Barbiturates can also lead to both psychological and physiological dependence. Benzodiazepines are commonly used but can lead to tolerance and physical dependence [5,6]. Ethanol, another CNS depressant, changes membrane fluidity and interacts with the GABA system, leading to tolerance and physical dependence [4,8]. Alcohol addiction affects 5% to 10% of men and 3% to 5% of women in American society [9]. It is important to find a natural CNS depressant with reduced or no toxicity. Medicinal plants have been utilized for thousands of years for various purposes, including food preservation, pharmaceuticals, alternative medicine, and natural therapies. Naturally produced compounds are considered more environmentally friendly as they can be biodegraded more easily than synthetic compounds[10].

Lannea coromandelica (Houtt.) Merr is a deciduous tropical tree found in India, Bangladesh, and other tropical countries, belonging to the Anacardiaceae family. Five dihydro flavanols were extracted and characterized from the stem barks of L. coromandelica[11]. The bark of L. coromandelica is beneficial for ulcers, wounds, ophthalmia, gout, sprains, diarrhoea, ulcerative stomatitis, and dysentery, while the leaves are beneficial for elephantiasis, inflammation, neuralgia, sprains, and bruises[12]. Additionally, the extract of *L. coromandelica* stem barks has been studied for its anti-inflammatory[13], hypotensive[14], and cytotoxic effects[15].

The present study examined the neuropharmacological and antidiabetic properties of the methanol and chloroform leaves extract of *L. coromandelica*. & results demonstrate the significance of the leaves for potential use in common mental illnesses related to depression as well as designed to stimulate specific antidiabetic targets.

2. MATERIALS AND METHOD:

2.1. Drugs and chemicals

Chloroform (S. D. Fine Chemicals Pvt) and methanol (S. D. Fine Chemicals Pvt.), all of LR grade, distilled under normal atmospheric pressure were employed for the extraction of the plant material.

2.2. Collection and extraction *of C. gigantea* leaves

Healthy and mature *Lannea coromandelica* plants were verified by ABS Medicinal Garden, Salem, Tamilnadu. after the leaves were gathered in May from the surrounding areas of Dharmapuri. When leaves from the highest portion of the plant were chosen and collected in the summer, a high phytochemical content was anticipated. During collection, care was taken to prevent contamination from dirt, dust, or other extraneous objects. To get rid of any contaminants, the gathered leaves were thoroughly cleaned in distilled water. After washing, the leaves were either spread out in a well-ventilated area to dry naturally or dried completely in a drying oven until they attained a consistent weight. To preserve consistency in particle size, the dried leaves were crushed into a coarse powder using a mortar and pestle or grinder. [24,32]

The dried and powdered plant material was weighed in a predetermined amount. Because it extracts a variety of phytochemicals with a moderate degree of polarity, ethyl acetate was selected as the extraction solvent. The Soxhlet extraction procedure, also known as maceration, was used to extract the material.[23] Throughout the Soxhlet extraction, ethyl acetate was constantly pumped through the dried and powdered material for several hours while it was contained in a thimble inside the Soxhlet extractor.

2.3. Animal

Wister albino mice (either sex) were bred at the Central Animal House, College of Pharmacy, the animals were allowed a standard pellet diet and water ad libitum. Groups of six mice (20-24 g) were used in all sets of experiments. The animals were fasted for 18 hours before use. The approval from the Institutional Animal Ethical Committee of Sri Vijay Vidyalaya College of Pharmacy was taken before carrying out biological studies.

3. Preliminary phytochemical Screening:

Preliminary phytochemical studies of the methanol and chloroform of *Lannea coromandelica* leaves aim to identify and characterize the chemical constituents present in this fraction. [24-26] Phytochemical analysis provides valuable insights into the potential bioactive compounds responsible for the medicinal properties of the plant.[32-36] Here are some common phytochemical groups that are often investigated:

***4. In vivo* pharmacological screening Assessment of anti-anxiety activity**

The neuropharmacological activities of ethyl acetate fraction extract of C. gigantea leaves were estimated by hole cross test, open field test, and elevated plus-maze (EPM) test. During each experiment, male Sprague Dawley rats were divided into three groups, namely, control, positive control, and test samples. Each group containing 5 mice was treated as the following arrangement: control, 1% v/v Tween-80 in water, 0.5 mL/mice; positive control, diazepam, 1 mg/kg body weight; test sample, ethyl acetate fraction extract at the dose of 400 mg/kg body weight.

4.1. Elevated Plus Maze Test (EPM)

The anxiolytic activity of plant extracts was evaluated using the EPM test. The apparatus was situated 40 cm above the floor, consisting of two open arms (5×10 cm) and two closed arms (5×10×15 cm) radiating from a platform (5×5 cm) to form a plus-sign figure. The open-arm edges were 50 cm in height to keep the mice from falling and the closed-arm edges were 15 cm in height. Sixty minutes after the administration of the test drug, each animal was placed at the center of the maze facing one of the enclosed arms. During the 5-minute test period, the number of open and enclosed arms entries, plus the time spent in open and enclosed arms, was recorded *via* the method of *Pillow and File*. Entry into an arm was defined as the point when the animal places all four paws onto the arm. The procedure was conducted in a sound-attenuated room; observations were made from an adjacent corner [28].

4.2 Open field test (OFT)

This test is one of the most frequently used methods to evaluate the locomotor activity and emotionality of rodents. The apparatus is a square box consisting of a 50 cm high wall and a wooden floor with a series of squares alternatively painted in black and white. Animals were administered with the vehicle, EAFCG, or diazepam and placed in the middle of the open field allowing free exploration. The animals were then scored with the number of squares they visited for 3 min before and at 30, 60, 90, and 120 min post treatments. The percentage of inhibition was calculated for each time point as described in the hole cross-test [29].

4.4 Measurement of free radical scavenging activity by DPPH assay:

The free radical scavenging activity of pyrazole derivatives was analyzed by using 2,2- diphenyl- 1-picrylhydrazyl (DPPH). The pyrazole derivatives solutions were prepared with various concentrations of about 100,200,300,400 and 500 µg/mL in DMSO. Ascorbic acid was used as positive control. Equal volumes of drug solution and DPPH solutions were mixed and kept incubated in a dark room for 30 min, and the absorbance at 517 nm was measured in triplicates. The DPPH scavenging effect was calculated as follows:

Scavenging effect (%) = (Control-sample O.D/control O.D) × 100

4.5 Statistical analysis

The results are presented as Mean ± SEM. The statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test, for these tests, One-way, two-way ANOVA followed by Bonferroni's post hoc tests was adopted. In all the cases $P < 0.05$ was considered significant.

5. RESULTS:

5.4. Preliminary phytochemical analysis

Freshly prepared crude extracts of *Lannea coromandelica* were qualitatively tested to detect the presence of various secondary metabolites of the plant including alkaloids, flavonoids, steroids, terpenoids, reducing sugars, tannins, anthraquinone, cardiac glycoside, and saponins were detected by the phytochemical tests (Table 1).

Tab 1: Qualitative analysis of the various phytoconstituents on the crude extracts of *Lannea coromandelica*

Substance	Alkaloids	Saponins	Tannins	Phenolics	Glycosides	Steroids	Essential oils	Carbohydrates	Flavonoids	Terpenoids
Methanol extract	-	+++	+	-	-	++	_	-	+++	+++
Chloroform extract	-	+++	++	++	-	++	--	-	+++	+++

+++ Prominently present. ++ Moderately present. + Slightly present. – Absent.

Table 2: Effect of Methanolic, Chloroform Extracts of *Lannea coromandelica* Leaves on Locomotor Activity (Actophotometer) in Mice at Different Time Intervals

Group	Treatment	Photocell count		% Change in activity	
		30 min	60 min	30min	60min
I	Control (vehicle)	302±2.320	319.7±2.482	NA	NA
II	Diazepam (5mg/kg i.p)	131*±6.852	92.36*±8.873	66.2	78.82
III	Methanolic extract(500 mg/kg p.o)	152.1*±2.124	112.33*±0.526	51.7	64.52
IV	Chloroform extract(500 mg/kg p.o)	112.7*±8.106	84.33*±0.546	24.5	33.82

*****- p<0.001, * - p< 0.05 compared to control**

Table 3: Effect of *Lannea coromandelica* on elevated plus maze apparatus

S.No	Group & Dose (mg/kg, i.p)	Mean No.of entries		Mean time spendin Sec.		Percentageof open arm entries	Percentageof time spent in open arm
		Open arm	Closed Arm	Open arm	Closed arm		
I	Control (vehicle)	4.5± 1.05	16.67± 2.241	62.67± 6.54	240.54± 9.54	25.34	21.45
II	Diazepam (5mg/kg i.p)	7.17± 0.82*	18.02± 6.052	98.83± 5.21*	221.71± 8.63	32.12	33.68
III	Methanolic extract (500 mg/kg p.o)	10.17± 0.75***	9.50± 2.34	142.50± 4.37***	178.50± 4.65	45.82	51.92
IV	Chloroform extract (500 mg/kg p.o)	14.50± 0.82***	10.0± 1.24	202.57± 4.91***	132.84± 6.37	61.23	68.12

Table.4: DPPH Assay of Extracts

Comp code	Concentraion (µg/ml)	Absorbance in (nm)	Mean Absorbance (nm)	Scavenging effect(%)
Methanolic extract	100	0.47	0.47	30.88
		0.48		
		0.47		
	200	0.45	0.45	33.82
		0.45		
		0.45		
	300	0.43	0.43	36.76
		0.42		
		0.43		
	400	0.41	0.41	39.70
		0.42		
		0.41		
	500	0.38	0.38	44.11
		0.38		
		0.38		
Chloroform extract	100	0.38	0.39	42.64
		0.39		
		0.39		
	200	0.38	0.38	44.11

DISCUSSION:

The anti-anxiety activity of various extracts was evaluated employing a widely used model, elevated plus-maze. The model was chosen as it is effective, cheap, and simple, less time-consuming, requires no preliminary training for the mice, and does not cause much discomfort to the animals while handling. The model is principally based on the observations that the exposure of animals to an elevated and open maze results in approach-avoidance conflict which is manifested as an exploratory-cum-fear drive.

The fear due to height (acrophobia) induces anxiety in the animals when placed on the elevated plus-maze. The ultimate manifestation of anxiety and fear in the animals is exhibited by a decrease in motor activity, which is measured by the time spent by the animal in the open arms. In mice of the control group is evident from the minimum mean time spent in the open arms of elevated plus-maze by these animals.

Among the extracts tested, maximum anxiolytic activity was observed in the methanol at the dose of 500 mg/kg which was at par with that of diazepam as is evident from statistical equivalence between the results of this dose and that manifested by diazepam. The average basal activity scores in the control group after 30 and 60 minutes of administration of Methanolic, chloroform extracts of *Lannea coromandelica* leaves 500 mg/kg p.o. significantly reduced locomotor activity. It may be due to the CNS depressant property of the drug.

Elevated plus maze test was used to assess the psychomotor performance and emotional aspects of rodents, treatment with Methanolic extract, and chloroform extract revealed anxiolytic activity since the number of open arm entries and time spent in open arm parameters are the most delegated guide for anxiolytic activity.

The time spent by the animal in the central platform seems to be related to decision-making or risk assessment and the total arm entries is a measure reflecting changes in anxiety.

In our study, we demonstrated significant anti-anxiety activity with 500mg of both extracts compared to the control group but had less activity when compared to that of diazepam but both were statistically significant.

As per the DPPH assay results it is found that Methanolic extract at 500 (µg/ml) showed a 44.11% Scavenging effect, and Chloroform extracts at 500 (µg/ml) showed a 51.47 % Scavenging effect. Standard ascorbic acid has an 80.88 % Scavenging effect

Recent evidence suggests a direct correlation between oxidative stress and anxiety. For example, Masood et al. (2008) reported that oxidative stress induced by L-buthionine- (S, R)-sulfoximine (BSO) in the hypothalamus and amygdala occurs in parallel with anxiety-like behavioral patterns in mice. Another study in outbred Swiss mice by Bouayed et al. indicated that increased anxiety-like behavior is positively correlated with increases in reactive oxygen species in granulocytes. Since there is a strong relationship between stress, neonatal handling and feeding behaviors, the influences of these three factors on behavioral parameters and oxidative stress in key brain regions have been investigated. These studies suggest that these factors are closely involved in behavioral activity, such as anxiety and locomotion, as well as redox components, such as ROS/SOD and NADPH levels [22-24].

In the present investigation, extracts showed moderate radical scavenging activity in the DPPH assay. This may be attributable to the high total phenolic and total flavonoid contents of the extracts. Qualitative phytochemical analysis showed that extracts contained a range of structurally diverse secondary metabolites, including alkaloids, tannins, phenols, and flavonoids. Previous studies have demonstrated that alkaloids, flavonoids, and phenols had anxiolytic activity owing to their high affinity for the benzodiazepine (BZD)-binding site of GABAA receptors.

Gamma-aminobutyric acid (GABA) is an important inhibitory neurotransmitter in the central nervous system (CNS). The binding of BZDs to GABAA receptors increases the opening of the linked chloride channel, leading to neuronal membrane hyperpolarization and anxiolytic activity. Other studies reported that plants rich in total phenolics and tannins could exert beneficial effects on anxiety and depression via upregulating the expression of GABAA and 5-HT1A receptors, serotonin, norepinephrine, dopamine, brain-derived neurotrophic factor, cAMP response element-binding protein, and reducing serum cortisol levels in animals [25-29].

SUMMARY AND CONCLUSION:

Anxiety and depression are the most common psychiatric disorders triggered by stress. The body has its defense mechanism, antioxidants, to counteract the biochemical changes caused by stress. In our daily lives, stress is inevitable and becomes a part of life's challenges, potentially manifesting as aging rather than clinical disorders. However, when stressors persist over time and begin to affect various aspects of

functioning such as mood, sleep, and appetite, they may indicate the presence of a disorder.

While there are various stress-induced psychiatric disorders, anxiety lies at the core, with generalized anxiety disorder (GAD) being the most prevalent presentation. Many individuals with GAD also experience symptoms of depression, and similarly, those with depression often exhibit anxiety symptoms. Additionally, a considerable number of patients suffer from a mixed condition known as mixed anxiety depression syndrome.

Recent research suggests a link between the imbalance of oxidative stress and the body's antioxidant defence system and the development of neuropsychiatric disorders like depression and anxiety. Specifically, major depression and anxiety are associated with a decrease in overall antioxidant levels and an increase in oxidative stress pathways. Traditional antidepressants may not only regulate neurotransmitters but also elevate antioxidant levels and mitigate the damage caused by oxidative stress processes, contributing to their therapeutic effects.

In our study, we demonstrated significant anti-anxiety activity with 500 mg of both extracts compared to the control group but had less activity when compared to that of diazepam but both were statistically significant. Both extracts showed a considerable amount of anti-anxiety when compared to diazepam 5mg/kg. Also, they were found to have anti-oxidant properties.

Conflict of interest statement

We declare that we have no conflict of interest.

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