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PHYTOCHEMICAL AND PHARMACOLOGICAL EVALUATION OF *CITRUS AURANTIFOLIA* (CHRISTM.) SW. STEM BARK EXTRACTS FOR ANTIDEPRESSANT ACTIVITY

Dhage Sadhana[1], Dr. S. K. Sarje[1], Shagufta Farooqui[1], A. V. Ingle*[2], Gaikwad Vishwata[1], Dr. N. B. Ghiware[1]

[1]Department of Pharmacology, Nanded Pharmacy College Nanded, Maharashtra, India-431605.
[2]Department of Pharmaceutical Chemistry, Nanded Pharmacy College, Shyam Nagar, Nanded- 431605.

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***Corresponding Author: A. V. Ingle**
Department of Pharmaceutical chemistry, Nanded Pharmacy College, Shyam Nagar, Nanded- 431605.
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ABSTRACT

The Phytochemical analysis of the extracts was carried out. Preliminary phytochemical evaluation of extracts was carried out for the determination of presence of phytoconstituents along with TLC. The medicinal plant *Citrus aurantifolia*(Christm.) Sw. belonging to the Rutaceae family, is renowned for its diverse biological activities and traditional medicinal uses. Extracts from its stem bark collected from Nanded, Maharashtra, India, were subjected to physicochemical characterization and evaluated for antidepressant activity. Various parameters such as ash value, extractive value, and solubility were assessed for the powdered drug. Extracts were obtained using pet-ether, acetone, and methanol via Soxhlet extraction. Phytochemical standardization, including TLC analysis, was performed to identify bioactive compounds. In-vivo antidepressant activity was evaluated using the Despair swim test and Tail suspension test in rats and mice, measuring immobility time. Methanol extract significantly reduced immobility time in both tests compared to the standard drug Imipramine, while acetone extract also showed notable activity. The findings suggest that *Citrus aurantifolia* stem bark extracts exhibit antidepressant potential, possibly attributed to the presence of flavonoids, phenolic compounds, and other phytoconstituents.

KEYWORDS: Citrus aurantifolia, Phytochemical analysis, Antidepressant activity, Thin layer chromatography.

INTRODUCTION

The study focuses on *Citrus aurantifolia* Swingle, commonly known as lime, a fruit-bearing medicinal plant from the Rutaceae family. Lime finds extensive use in cosmetics, food flavoring, beverages, and traditional medicine due to its

diverse biological properties, including anti-cancer, antimicrobial, antioxidant, and anti-inflammatory effects. Its medicinal significance stems from a rich array of secondary metabolites such as alkaloids, flavonoids and phenolic compound. Despite its extensive use in various ailments ranging from cold fevers to inflammatory bowel disease, there's a lack of scientific data on the antidepressant effects of *Citrus aurantifolia* stem bark. Therefore, the present study aims to fill this gap by evaluating the phytochemical and pharmacological aspects of *Citrus aurantifolia* stem bark extract for its antidepressant activity, supporting its traditional medicinal claims.

MATERIALS AND METHODS

The physical evaluation of dried stem barks involves determining several physicochemical parameters such as total ash value is determined by igniting 2g of the powder in a porcelain dish, burning off carbon, and weighing the residue. Acid-insoluble ash value involves treating the total ash with dilute hydrochloric acid, filtering, igniting, and weighing the residue. Water-soluble ash value is found similarly, but the ash is washed with water instead.

Loss on Drying (LOD) is determined by weighing 2g of the sample, drying until consecutive weighing’s don't differ by more than 0.5mg, then recording the weight loss as moisture. Extractive values are determined for both water and alcohol solubility. For water-soluble extractive values, 5g of air-dried drug is macerated with distilled water, filtered, dried, and weighed. Alcohol-soluble extractive values are obtained similarly, but using ethanol instead. These procedures provide essential data for assessing the quality of the stem barks.

3.1 Extraction of plant material:

The extraction process detailed in the article focuses on deriving valuable compounds from the stem bark of *Citrus aurantifolia* (Christm.) Sw. The selection of solvents, including petroleum ether, acetone, and methanol, was guided by extractive value assessments and an understanding of the plant material's phytochemical composition. Following a literature review, the Soxhlet extraction method was identified as optimal, given its efficiency and suitability for extracting heat-stable constituents. The method involved defatting the powdered stem bark in a Soxhlet extractor, followed by extraction with the chosen solvent. Once the extraction was complete, the resulting extracts were cooled, dried, and stored securely. The percentage yield of each extract was meticulously calculated, providing quantitative insights into the extraction efficiency. This systematic approach ensures the production of high-quality extracts, laying a robust foundation for further analysis and research applications Petroleum ether extract was obtained by continuous hot extraction in a Soxhlet apparatus. 270 gm of powdered stem bark was extracted with 1500 ml of petroleum ether. Acetone and Methanol extracts were similarly prepared using 267 gm of powdered husk and 265 gm of powdered pods, respectively, with corresponding solvents. After extraction, each extract was cooled, dried, and stored for future use. Percentage yield of each extract was calculated to assess extraction efficiency. This approach ensured the extraction of various bioactive compounds for further analysis and potential applications.

3.2 Phytochemical Analysis

The phytochemical analysis of the extracts from the stem bark of *Citrus aurantifolia* (Christm.) Sw. revealed a diverse array of chemical constituents. Carbohydrates were detected in acetone and methanol extracts through positive results in Fehling's and Benedict's tests. Protein presence was confirmed in the pet ether and methanol extracts via positive Biuret tests. Steroids were found in the pet ether and methanol extracts, indicated by positive Salkowski tests.

Glycosides were detected in acetone extracts, as shown by positive Baljet and Keller-Killiani tests, while saponin glycosides were present in both acetone and methanol extracts, evidenced by foam formation. Flavonoids were abundant in all extracts, with positive results in Shinoda, lead acetate, and zinc dust + HCl tests. Tannins and phenolic compounds were notably present in acetone and methanol extracts, indicated by positive results in lead acetate, 5% ferric chloride, and dilute iodine solution tests. Alkaloids were detected in acetone and methanol extracts, confirmed by positive results in Wagner's and Mayer's tests, among others. Overall, the comprehensive phytochemical analysis underscores the rich chemical diversity within the *Citrus aurantifolia* stem bark extracts, providing valuable insights into their potential pharmacological properties and applications.

3.3. Pharmacological Screening of plant extracts

In-vitro antioxidant activity assesses a substance's ability to inhibit oxidation, crucial in preventing cell damage caused by free radicals. *Citrus aurantifolia (Christm.) Sw*antioxidant activity was evaluated using the DPPH free radical scavenging assay. This method measures the ability of antioxidants to neutralize DPPH radicals, indicative of their antioxidative potential. Test solutions of *Citrus aurantifolia* (Christm.)Swstem bark extracts (Acetone and Methanolic) were prepared at various concentrations, ranging from 20µg/ml to 100µg/ml. Similarly, standard solutions of Quercetin were prepared for comparison. The assay was conducted in replicates, and the average results were considered for analysis. By determining the scavenging activity of the test samples compared to the standard, the antioxidant potential of *Citrus aurantifolia* (Christm.) Swextracts were evaluated. This method provides valuable insights into the antioxidative properties of natural compounds, aiding in the development of potential therapeutic agents against oxidative stress-related disorders.

The DPPH radical scavenging assay is a widely utilized method to evaluate the antioxidant activity of compounds. It operates on the principle of reducing the stable free radical DPPH in a methanol solution, resulting in the formation of the non-radical form DPPH-H. This reaction causes a color change from purple to yellow, which is detectable spectrophotometrically at 517 nm. The disappearance of the purple color indicates the scavenging of free radicals by the antioxidant compounds being tested. In the procedure, a reaction mixture comprising 1 ml of 0.1 mM DPPH solution in methanol, 1 ml of the drug solution, and 1.0 ml of methanol is prepared. After vertexing, the mixture is incubated in the dark at room temperature for 30 minutes. Subsequently, the absorbance is measured at 513 nm, with a control reaction mixture lacking the test sample serving as a reference. This assay provides valuable insights into the antioxidant potential of substances, aiding in various applications, including pharmaceuticals, food science, and cosmetics.

3.4. Dose Considerations

Acute toxicity studies conducted by many researchers for *Citrus aurantifolia* stem bark extracts as per standard references revealed that the administration of graded doses extracts (up to a dose of 2000 mg/kg) did not produces any significant changes in behaviors such as alertness, motor activity, breathing, restlessness, diarrhea, convulsions, coma and in appearance of the animals. No death recorded up to the dose of 2000 mg/kg body weight. The animals were physically active. The results of such studies showed that in single dose; the plant extracts had no adverse effect, indicating that the medium lethal dose (LD 50) could be greater than 2000 mg/kg body weight in experimental small animals as in rats/mice. Accordingly safe experimental dose considered for present investigation as ≤2000 mg/kg.

3.5. In-vivo study

The antidepressant activity of a substance was evaluated using two established behavioral tests: the Forced Swimming Test (FST) and the Tail Suspension Test (TST).

In the FST, rats were individually placed in a cylindrical container filled with water and their immobility duration was recorded during the last 6 minutes of a 10-minute observation period. Immobility was defined as floating motionless with minimal limb movements to keep the head above water. The study included six groups: a control group receiving CMC (2%) in saline water, a standard group receiving Imipramine, and a drug-treated group divided into two subgroups receiving different doses of the test substance. Changes in immobility duration were analyzed to assess antidepressant effects.

In the Tail Suspension Test, mice were individually suspended by their tails, 50 cm above the floor, using adhesive tape placed near the tail tip. The total duration of immobility during a 6-minute observation period was recorded. Immobility was characterized by passive hanging or complete motionlessness. Antidepressant-like activity was indicated by a reduction in immobility time. This test is widely used to assess antidepressant effects in mice and is based on the concept that brief, inescapable stress induces an immobile posture.

RESULT

4.1 Pharmacognostic evaluation of *Citrus aurantifolia* (Christm.) Sw. stem bark

The determination of physicochemical properties of *Citrus aurantifolia* (Christm.) Sw Stem Bark powder involved analyzing extractive values and ash content. In extractive value analysis, various solvents were used, with methanol showing the highest extractive value at 5.25% followed by acetone at 3.25%.

The ash content was assessed in three forms: total ash, acid insoluble ash, and water-soluble ash. Total ash content was found to be 11.5%, while acid insoluble ash and water-soluble ash values were 3.5% and 2%, respectively. Loss on drying, indicating moisture content, was determined to be 11%.

Additionally, phytochemical evaluation involved extracting *Citrus aurantifolia* (Christm.) Sw.with different solvents, yielding different percentages of extract, with Acetone extract showing the highest yield. These findings provide insights into the chemical composition and potential pharmacological properties of *Citrus aurantifolia* (Christm.) Sw. Stem Bark extract.

4.2 Phytochemical tests of *Citrus aurantifolia* (Christm.) Sw.stem bark extract

Table No. 1: Observations for Phytochemical qualitative analysis.

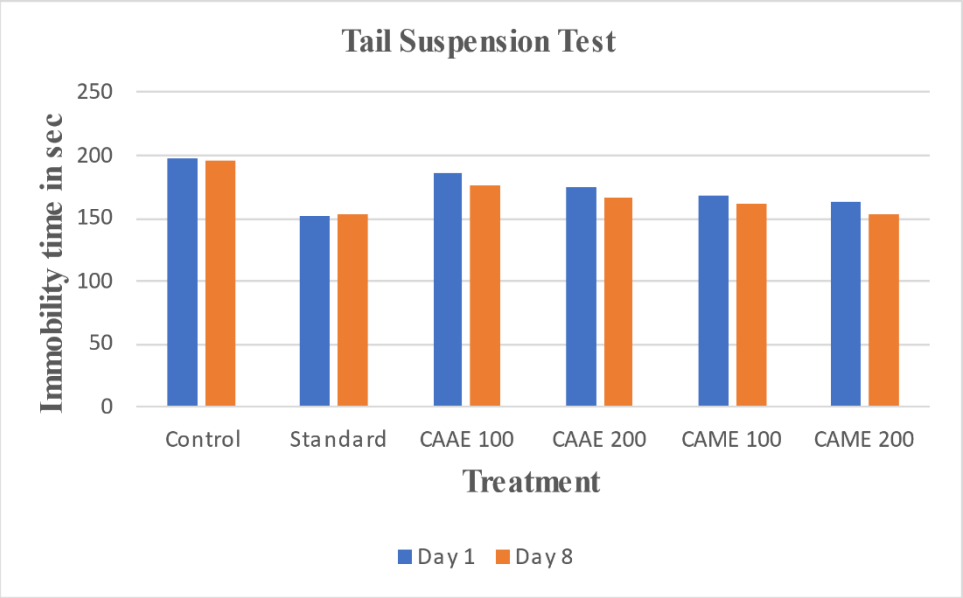
Chemical test	Pet ether	Acetone`	Methanol
Test for Carbohydrate			
Molisch test	-	+	+
Fehling's test	+	+	+
Benedict's test	-	+	+
Barford's test	-	-	-
Test for Proteins			
Biuret test	+	-	+
Millon's test	-	+	-
Test for amino acid			
Ninhydrin test (general test)	-	-	-

Test for Steroid			
Salkowski test	-	+	+
Liebermann Burchard reaction	-	-	-
Test for Glycosides			
Baljettest	-	+	+
Keller-killiani test	-	+	+
Test for saponin Glycosides			
Foam test	+	+	+
Test for Flavonoids			
Shinodatest	+	+	+
Lead acetate test	+	+	+
Zinc dust +HCL test	-	-	+
Test for Tannins and phenolic compound			
Leadacetate test	+	+	+
5%Ferricchloridetest	-	+	+
Dil.iodine solution	-	+	+
Bromine water	-	-	-
Test for Alkaloids			
Dragendroff's test	-	-	-
Wagner's test	+	+	+
Mayer's test	+	-	-
Hager's test	-	+	+
Tannic acid test	+	+	+

4.4. Tail Suspension Test model

Table No. 3: (Effect of Day 8 CAAE and CAME (100 & 200 mg/kg) on Immobility time in TST model on mice)

Treatmentgroup	Tail suspension test (Immobility time in sec)	Tail suspension test (Immobility time in sec)
	Day 1	Day8
Imipramine10 mg/kg	152**	152.83±1.6**
Control	197.34	196.16±1.95
CAAE100mg/kg	186.45	177±1**
CAAE200mg/kg	175.23**	166.5±1.91**
CAME100mg/kg	168.48**	160.83±1.27**
CAME200mg/kg	162.36**#	153.66±1.28*#



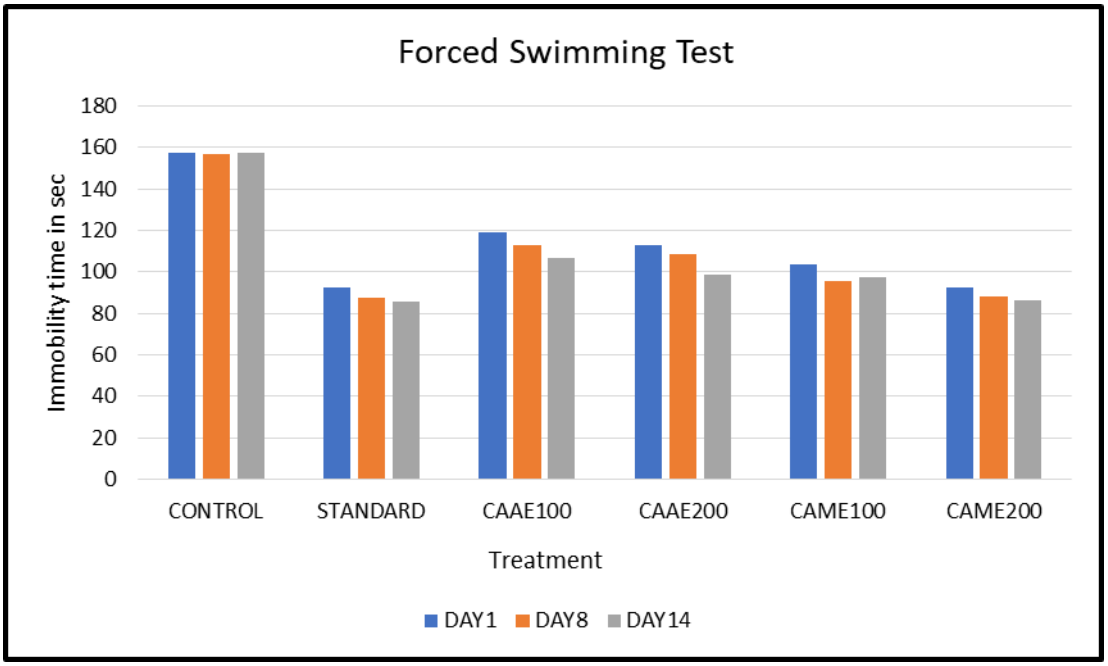
(Effect of *Citrus aurantifolia* and Imipramine (10 mg/kg) on day 1 & 8 TST. (The column represents mean duration of immobility recorded in a 6 min observation period.)

Values are expressed as mean ± SEM (n = 6), *: significant difference (P< 0.05 or less) & **: highly significant difference (p <0.001) when compared with control, (One way ANOVA followed by Tukey's test.) #p>0.05 non-significant difference when compared with standard

4.5. Forced Swimming Test model

Treatment group	Forced Swimming Test (Immobility time in sec)		
	Day 1	Day8	Day14
Imipramine 10mg/kg	92.33±1.62**	87.66±1.4**	85.33±1.22**
Control	157±2.54	156.5±2.51	157±2.54
CAAE100mg/kg	118.83±1.27**	112.66±1.4**	106.33±2.01**
CAAE200mg/kg	112.83±0.1.42**	108.66±1.2**	98.5±1.38**
CAME100mg/kg	103.66±1.38**	95.33±1.05*	97.5±2.14**
CAME200mg/kg	93.16±1.53**#	88±1.59**#	86.83±1.1**#

Values are expressed as mean ± SEM (n = 6), *: significant difference (P< 0.05 or less) & **: highly significant difference (p <0.001) when compared with control, (One way ANOVA followed by Tukey's test.) #p>0.05 non-significant difference when compared with standard.



(Effect of *Citrus aurantifolia* and Imipramine (10mg/kg) on day 1 & 8 TST. (The column represents mean durationofimmobilityrecordedina6minobservationperiod.)

SUMMARY AND CONCLUSION

Citrus aurantifolia (christm.) sw. stem bark was used for studying pharmacogenetic, phytochemical and pharmacological evaluations.

The study investigates the antidepressant potential of crude extracts obtained from *Citrus aurantifolia* (christm.) sw. stem bark, focusing on pharmacognostic, phytochemical, and pharmacological evaluations. Two extracts, CA acetone and CA methanol, are prepared and subjected to analysis, revealing the presence of various bioactive compounds like carbohydrates, alkaloids, glycosides, flavonoids, saponins, steroids, tannins, and phenolic compounds. In vitro antioxidant assays confirm the extracts' significant antioxidant activity, suggesting their potential in combating diseases linked to oxidative stress. Evaluation through Forced Swimming Test and Tail Suspension Test on rats and mice demonstrates notable antidepressant effects, with both extracts showing significant reductions in immobility time compared to controls and standard treatment with Imipramine. Particularly, the methanol extract exhibits more significant antidepressant activity than the acetone extract. These findings support the notion that *Citrus aurantifolia* (christm) sw. stem bark extracts could serve as herbal remedies for depression.

The finding of the present study demonstrated that *Citrus aurantifolia* (christm) sw. stem bark extracts has potent anti depressant potential and justify its use in traditional medicine to treat.

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