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PHARMACOGNOSTIC EVALUATION, PHYTOCHEMICAL SCREENING AND PHARMACOLOGICAL ACTIVITIES OF *ZEPHYRANTHES CITRINA*

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ABSTRACT

The *Zephyranthes citrina* has not yet been investigated and reported for its pharmacological properties. This study investigates the Pharmacognostic study, phytochemical screening and physicochemical analysis of dried whole plant of *Z. citrina*. Acute toxicity was performed on Wister albino rats which was found to be 1000 mg/kg of body weight. The plant was assessed for its antioxidant potential. Ascorbic acid was used as standard drug and plant extract showed marked results. Methanolic extract of the plant was also investigated for its anti-inflammatory and analgesic activities at the dose of 100 mg/kg and 200 mg/kg. Diclofenac sodium at the dose of 50 mg/Kg and Tramadol at the dose of 30 mg/Kg were used as standard drugs respectively. These effects were found to be dose dependent and analgesic effect lasts for 120 minutes at the dose of 100 mg/kg and 200 mg/kg.

Keywords: Pharmacognostic, phytochemical, physicochemical, antioxidant, anti-inflammatory, analgesic

INTRODUCTION

In different regions of Pakistan people have century's old information and conventional practice to use medicinal plants found in their areas. [1]. Yellow Rain Lily is also known as *Zephyranthes citrina*, *Zephyranthes sulphurea*, *Zephyranthes eggersiana*, *Citron*

Zephyr lily, Yellow Zephyr lily. Yellow Rain Lily, is a clump-developing bulbous perennial plant (monocotyledonous family Amaryllidaceae) from the Yucatan Peninsula in Mexico. Its rush-like leaves length is normally 12 inches. It has small, deep yellow flowers. It comprises almost 90 species, of which about 37 are innate to Mexico, many of which are endemic [2].The genus *Zephyranthes* contain many diverse species generally called as rain lilies or surprise lilies due to their convention of periodic flowering after rainfall season. [3]. Natural plants having great significance in the battle as compared to invasive plants that intimidate natural environments all over the country. Natural species meet and beat standards of deficiency, ailment, and also pest tolerance in their natural habitats, however at the same time providing help in the colors, consistencies, and shapes that makes landscape projects successful [4]. The methanolic extract of dried leaves of *Zephyranthes candida*was reported to have antibacterial activity against *Styphylococus aereus, E. scherichia coli, Bacillus subtilis, Pseudomonas, Klebsiella pneumonia and Entero bacteraerogenes* bacterial strains by using Agar well diffusion method [5]. *Zephyranthes citrina* contains eight alkaloids like lycorine, haemanthamine, galanthine and lycorenine that can be easily isolated from *Zephyranthes citrina* [6]. Antifungal properties were also shown by two alkaloids isolated from bulbs of *Zephyranthes citrina* [7].

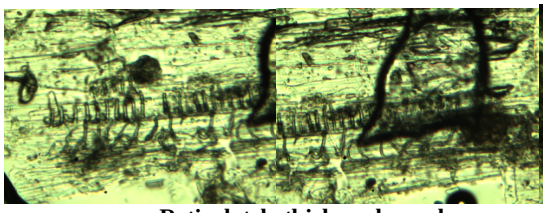
RESULTS AND DISCUSSIONS

Macroscopic Evaluation: *Z. citrina* contains yellow color flowers and the color of plant is green. The *Z. citrina* have characteristics order, cellulose like taste and smooth texture.

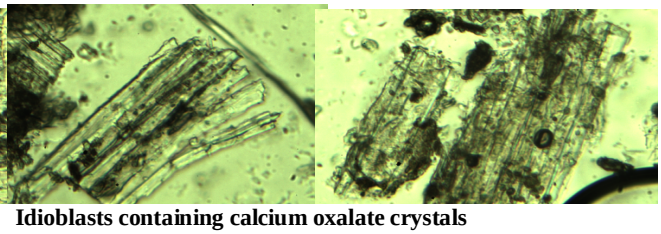
Microscopic features: The powder microscopy of *Z. citrina* showed that the plant contained reticulately thickened vessels, idioblasts containing calcium oxalate crystals, epidermis and covering trichomes.

Moisture content: Moisture content of *Z. citrina* was found to be 1.08% which is less than 10%.

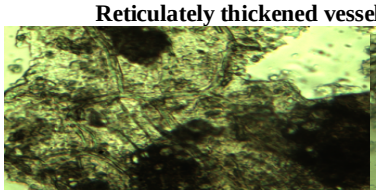
Fluorescence Analysis: Results of fluorescence analysis are shown in table 2. For first line standardization of crude drug, fluorescence study is important. Wavelength of fluorescent light is always greater than exciting light in fluorescence analysis. Fluorescence can be actively produced in by the light of short wavelength therefore in many substances ultraviolet light is used to produce fluorescence which does not gives visible fluorescence in day light [8].



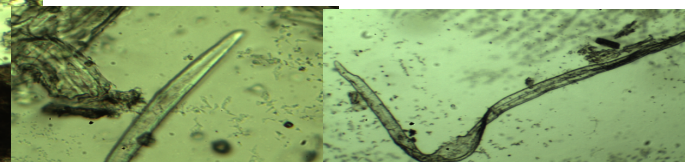
Reticulately thickened vessels



Idioblasts containing calcium oxalate crystals



Epidermis



Covering trichomes

Phytochemical screening:

Table 1: Preliminary Phytochemical screening

Constituents	Observations
Alkaloids	
Mayer's test	+
Wagner's test	+
Hager's test	+
Glycosides	
Legal test	–
Killer-killani test	+
Phenolic compounds and Tannins	
Ferric chloride test	+
Lead acetate test	+
Gelatin test	–
Terpenoids	–
Carbohydrates	
Molish test	+
Fehling test	+
Barfoed's test	+
Catechin	+
Gums	–
Mucilages	–
Saponins	+
Steroids	+
Test for Proteins and Amino acids	
Ninhydrin test	–
Biuret's Test	–

Table 2: Fluorescence Analysis of Dried Powder of *Z. citrina*

Reagents	Visible light	Short UV(254nm)	Long UV(366nm)
HCl	Dark brown	Blue	Blue
Distilled water	Light brown	Reddish blue	Greenish blue
Methanol	Yellow	Reddish blue	Pinkish white
NaOH	Dark brown	Purple	Blue
Ammonia	Orange	Pinkish blue	Green
H_2SO_4	Black	Purple	Blue
Chloroform	Brown	Greyish blue	Pinkish blue

Physico-chemical evaluations:

Total Ash value: Total ash value of *Z. citrina* was found to be 09%. Generally ash value (total ash) shows the existance of inorganic salts e.g., drugs contain calcium oxalate naturally and organic matter can be obtained from external source [9].

Acid insoluble ash: The acid soluble ash value of *Z. citrina* is 4.50 %.

Water insoluble ash: Water soluble ash of *Z. citrina* is 5.00%. Samples which have been extracted with water can be easily detected by water soluble ash [9].

Foaming index: If the height of foam in every test tube was more than 1 cm then the foaming index was more than 1000. By using the formula result was obtained

Foaming index = 1000/ a

a = decoction volume in ml was used for the preparation of dilution in every test tube. So the foaming index is 103.63.

Antioxidant Activity: The antioxidant activity of Methanolic extract of *Z. citrina* was more than that of Ascorbic acid when taken at 517 nm as shown in table 3. Antioxidant activity may be due the presence of alkaloids, glycosides, tannins, phenolic compounds, carbohydrates, proteins, saponins, gums, terpenoids, flavonoids and steroids as following medicinally active plants showing antioxidant activity contains these chemical constituents e.g., Fennel and Ajwain (*Trachyspermum ammi*) have alkaloids, glycosides, tannins, flavonoids and saponins[10].

Acute Toxicity Test: The methanolic extract of *Z. citrina* showed mortality at the dose of 1000mg/kg and all the rats were died at this dose.

Anti-inflammatory Activity: Different test doses of 100 and 200 mg/kg having anti-inflammatory potential of methanolic extract of *Z. citrina* is arranged in table no. 4 with the average paw thickness of rats. The standard drug diclofenac sodium at the dose of 50mg/kg showed more distinct inhibition potential than the extract at the doses of 100 and 200mg/kg. The above mentioned results showed the dose dependent relationship by increasing the dose the %age inhibition also increased. Alkaloids are most effective medicinally important plant constituents. Alkaloids shows anti-inflammatory and analgesic activities. For example *Strychnosnux-vomica* [11], *Phyllanthus amarus* and *Phyllanthus fraternus* [12], *Moringa oleifera* [13], Kupeelu seeds [14], Morphine-6-glucuronide [15] and *Phyllanthus fraternus* [16].

Table 3: Antioxidant activity of Methanolic extract of *Z. citrina*

Conc. of Extract/ Standard(µg/ml)	Absorbance of MEZC	Inhibition effect (%)	Absorbance of ASCA	Inhibition effect (%)
125	0.05 ± 0.000882	72.37	0.053 ±0.000577	70.72
250	0.054 ±0.001732	70.16	0.054 ±0.000667	70.16
500	0.066 ±0.000882	63.53	0.055 ±0.000577	69.61
1000	0.077 ±0.000333	57.46	0.057 ±0.000667	68.51

MEZC= Methanolic extract of *Z. citrina* ASCA= Ascorbic acid

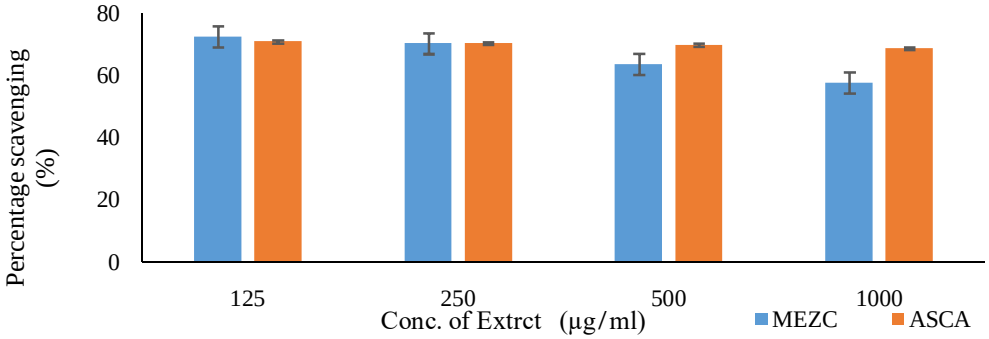
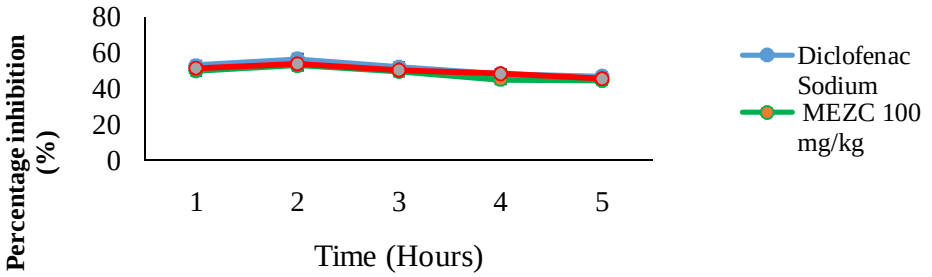


Table 4: Effect of *Z. citrina* in carrageenan induced rat paw edema

Treatment	Dose	Normal paw size (mm)	Paw size (mm) after administration of plant extract				
			1 Hour	2 Hour	3 Hour	4 Hour	5 Hour
Normal saline	10ml/kg	3.19±0.090	3.85±0.042	4.25±0.085	4.40±0.077	4.10± 0.049	4.00±0.062
Diclofenac sodium	50mg/kg	3.09±0.042	3.40±0.169*	3.55±0.159**	3.67±0.179**	3.56±0.138*	3.52±0.133*
MEZC	100mg/kg	3.15±0.044	3.48±0.138	3.65±0.123**	3.76 ±0.161*	3.65±0.134*	3.60±0.136
MEZC	200mg/kg	3.18±0.045	3.50±0.079	3.67±0.090**	3.78 ±0.171*	3.65±0.138*	3.62 ±0.137

MEZC: Methanolic extract of *Z. citrina.* Values are reported as mean ± S.E.M. for group of five animals. The data was analyzed by ANOVA. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, ***P<0.001



Analgesic activity: Different test doses of 100 and 200mg/kg of methanolic extract of *Z. citrina* having analgesic potential are arranged in table 5 with the average latency time. Tramadol was given orally at the dose of 30mg/kg that produced analgesic effect. In comparison to negative control group the positive control group, extract groups (100mg/kg and 200mg/kg) exhibited significant analgesic effect after 60 minutes, while after 90 minutes the results were highly significant which remained till 120 minutes.

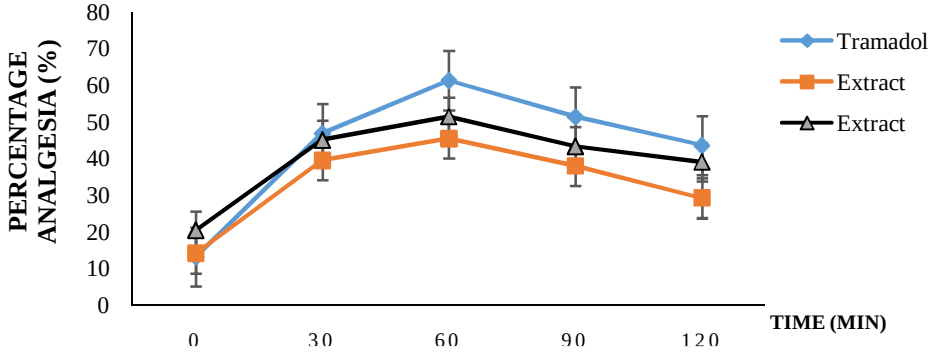


Table 5: Analgesic effects of methanolic extract of *Z. citrina*

Treatment	Dose	Latency time				
		Normal	30 Min	60 Min	90 Min	120 Min
Normal saline	10ml/kg	5.58 ± 0.269	5.61 ±0.220	5.58± 0.109	5.58 ± 0.112	5.57 ± 0.147
Tramadol	30mg/kg	6.32 ± 0.862	8.24 ± 1.61	9.00±1.315**	8.45±0.152***	8.00± 0.072***
Extract	100mg/kg	6.37 ± 1.281	7.83±0.459	8.12± 0.390*	7.70±0.365***	7.20± 0.355***
Extract	200mg/kg	6.72 ± 0.471	8.14±0.116	8.45± 0.143*	8.00±0.083***	7.75± 0.082***

MEZC: Methanolic extract of *Z. citrina.* Values are reported as mean ± S.E.M. for group of five animals.The data was analyzed by ANOVA. Asterisks indicated statistically significant values from control. *P<0.05, **P<0.01, ***P<0.001

MATERIALS AND METHODS

Collection of plant material:

The plant was collected from the botanical garden of Govt. College University Lahore. The specimen of the *Z. citrina* vide voucher no. GC.herb.bot.2424 was authenticated by Prof. Dr. Zaheer ud Din Khan, Herbarium Department of Botany, GC University Lahore.

Extraction of plant material: The extraction of plant material was carried out under standard conditions [17].The whole plant of *Z. citrina* was cleaned and dried under shade and powdered in mechanical grinder. 1 kg powder was macerated with methanol for 7 days with irregular shaking at room temperature. It was then filtered by a two folds covered muslin cloth and subsequently by a Whatman No.1 filter paper. Then the collected filtrate was concentrated in vacuum under reduced pressure by using a rotary flask evaporator (IKA HB 10 basic) accompanied with IKA KV-600 digital recirculation chiller, a water bath model IKA RV 10 basic at 40^{o}C and vacuum pump model MPC 105 T ILMVAC, to form a dense, semi-solid mass which is called as crude methanolic extract. The extract was then kept in sterile bottles under refrigerated conditions (2-4 ^{0}C) for future use.

Pharmacognostic features:

Macroscopic Evaluation: The powder of dried whole plant of *Z. citrina* was subjected to macroscopic a study which covers the organoleptic characteristics like odor, color, taste, appearance, shape etc. [18]

Microscopic Evaluation: The microscopic evaluation of dried powder of *Z. citrina* was done by using the digital microscope connected with computer system (Olympus Mic-D). The dried powder of whole plant of *Z. citrina* was cleaned in chloral hydrate solution. After the completion of cleaning process, the specimen was mounted on microscopic glass slide with glycerin. Then kept cover slip on it and observed under microscope. [19].

Phytochemical screening: The procedure was adopted for qualitative phytochemical analysis as described by [20]. Different phytochemical tests were performed on

methanolic extractfor the presence of different chemical constituents.

Physico-chemical evaluations Moisture content: 2 g powder of *Z. citrina* was taken in evaporating china dish which was previously dried and weighed. A preheated oven at 105 ^{0}C was used to dry this powder until it gave a constant weight up to 3 continuous readings. The loss on drying was noted and on the basis of powder sample the %age yield was calculated.The moisture content was calculated by using the following formula: [21]

$$\%\text{age of moisture content} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

Fluorescence Analysis: Fluorescence analysis of dried powder of *Z. citrina* was done according to standard procedure. In fluorescence analysis the dried powder was treated with different basic and acidic reagents and solvents. Then observed in a chamber exposed to UV/visible under different regions such as visible, short and long wavelength simultaneously. The variations in color and shape (appearance) were observed and noted [22]**.** Change in color was noted generally in reagents such as dried powder was added in aqueous 1N NaOH, in HCl, in distilled water, in Methanol, in Ammonia solution, in H_2SO_4, in Chloroform and in $FeCl_3$ [23].

Total Ash value: About 2 g of dried powder of *Z. citrina* that was weighed in a tared china dish and incinirated at a temperature greater than 450℃ until carbon was removed. Then cooled in desiccator. Carbon free ash was mixed with water, on the ash less filter paper the filterate (residue) was obtained. Then ignited the residue and filter paper at lower temperature to achieve constant weight. Then again cooled in desiccator and weighed [9]. The %age yield of total ash was calculated with reference to dried powder of *Z. citrina* by the following formula

$$\%\text{age of total ash} = \frac{\text{Weight of total ash}}{\text{Weight of sample taken}} \times 100$$

Acid insoluble ash: In the obtained ash (total ash) added 25 ml of dil HCl and boiled for 5 minutes. On the ash less filter paper the filterate (residue) was obtained. Then washed and ignited at lower temperature to achieve constant weight. Then again cooled in desiccator and weighed.The %age yield of acid insoluble ash was calculated with reference to dried powder by the following formula:

$$\%\text{age of acid insoluble ash} = \frac{\text{Weight of acid soluble ash}}{\text{Weight of sample taken}} \times 100$$

Water insoluble ash: Added 25 ml water in the obtained total ash and boiled for 5 minutes. On the ash less filter paper the insoluble substances were obtained. Then

washed with water and ignited at lower temperature to achieve constant weight. Then the weight of insoluble substance was subtracted from the total ash weight. %age yield of water soluble ash was calculated with reference to dried powder by the given formula:

$$\%\text{age of water soluble ash} = \frac{\text{Weight of total ash} - \text{weight of soluble ash}}{\text{Weight of sample taken}} \times 100$$

Foaming index: 1 g of dried powder of *Z. citrina* was dissolved in a conical flask having boiling water of 100ml, the prepared decoction was filtered. Then the decoction was transferred into ten test tubes (stoppered) having 16 cm height and 16 mm diameter in sequential ratio of one ml, two ml, three ml etc. up to ten ml and in each test tube the volume of solvent (liquid) was made up to 10 ml with distilled water. For 15 seconds the stoppered test tubes were shaken vertically. These test tubs were kept for 10- 15 minutes and measured the formation of foam (height) [24].

Antioxidant Activity: DPPH radical scavenging activity was determined by using various fractions of plant methanolic extract of *Z. citrina* by adopting reported method. Ascorbic acid was used as standard. In order to prepare 0.1mM DPPH solution, 0.04g of DPPH was dissolved in 1L of methanol. 0.02g of extract and sample was dissolved in 20ml methanol to prepare the 1000µg /ml stock solution of extract and sample.Different solution of 125 µg/ ml, 250 µg/ ml, 500 µg/ ml and 1000 µg/ ml were prepared from the stock solution.In 3mL of DPPH, previously prepared above stock solution and dilutions were mixed and shaken vigorously. The mixture was allowed to stand for 60 minutes at room temperature. After the specified time, the absorbance of the above mixture was measured in the spectrophotometer at 517 nm by taking Methanol as a blank [25][26].At the lower absorbance of spectrophotometer, higher free radical scavenging activity was observed.The following formula was used to calculate the scavenging of free radical.

$$\text{Percentage scavenging (\%)} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Experimental Animals: For *in-vivo* analysis of *Z. citrina*, Wister albino rats (male and female) both sexes were chosen having weight 200 to 250 g which were brought from the animal house of the University of Lahore. All the animals were kept under standard laboratory environment keeping temperature at 25 ^{0}C. Animals were kept in polyvinyl cage and maximum 5 animals in one cage. They were kept in this temperature for twelve hour dark/light and given feed and water *ad libitum* (freely).

Acute Toxicity Test: According to OECD-423 guidelines, acute oral toxicity study was executed. All rats were divided into three groups containing three rats in each group. The animals were being given nothing for eating except water and then they were given extract orally at the dose level of 100mg/kg and for 24 hours they were under observations. If 2 or 3 animals were died then dose was considered toxic. But if 1 animal died then the same dose was repeated for the confirmation of toxicity. If animals did not die then same procedure was repeated for higher dose level such as 200, 400, 800 and 1000mg/kg.

Anti-inflammatory activity: Albino rats of either sex weighing 200-250g were distributed into four groups, each group containing five rats: Group 1was given normal saline orally at the dose of 10mg/kg body weight. Diclofenac sodium was given orally to group 2 at the dose of 50mg/kg body weight[27]. Methanolic extract of *Z. citrina* was given orally to the group 3 and 4 at the dose of 100 and 200 mg/kg body weight respectively. 1% DMSO was used as a vehicle for extract. After these drugs each rat was given sub planter region injection of 0.05ml of 1% carrageenan in its right hind paw. Before given injection rat's paw size was measured by Vernier caliper [28]. It was also noted after 1, 2, 3, 4 and 5 hours after carrageenan injection.The %age inhibition was calculated by using the following formula: [29]

$$\text{Inhibition (\%)} = 1-[(a-x) / (b-y)] \times 100$$

Where

a = mean paw size of treated rats after carrageenan injection

x = mean paw size of treated rats before carrageenan injection

b = mean paw size of control rats after carrageenan injection

y = mean paw size of control rats before carrageenan injection

Analgesic activity: The rats of either sex (n=5) of weight 200-250 g were taken, then these rats were subjected to pre-testing by using hot plate (Havard apparatus). The temperature of hot plate was maintained at 55±0.1 ^{0}C. Those rats were rejected that were having latency time greater than 15 second on hot plate. Four groups of rats were made: group I given Normal saline. Group II was given Tramadol at the dose of 30mg/kg. Group III and IV was given methanolic extract of *Z. citrina* orally with 100 and 200 mg/kg of body weight. After 30 min of dosing rats were put on hot plate and their latency time was recorded in seconds. In order to avoid the tissue damage a cut off time of 30 seconds was enforced in all rats [30]. Percentage analgesia was calculated by using the subsequent formula:

$$\text{Analgesia (\%)} = \frac{\text{Treated latency- Control latency}}{\text{Control latency}} \times 100$$

CONCLUSIONS

The phytochemical screening of methanolic extract of *Z. citrina* reveals that alkaloids, glycosides, tannins, carbohydrates, saponins, steroids, catechin and phenolic compounds are present while gums, mucilages, proteins and terpenoids are absent. Lycorine (alkaloid) also shows the anti-inflammatory, anti-tumor, antiviral, analgesic, cholinesterase activity, hypotensive, antimalarial, antifeedant, antiplatelet, antifertility and antiarrhythmic activities. Lycorine is active constituents of *Z. citrina* [31].

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