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Organoleptic, fluorescence, qualitative and quantitative analysis of bulb extract of *Zephyranthes citrina*

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Abstract
Phytochemicals are extensively found at different levels in many medicinal plants. Our current research investigation is an attempt to assess the organoleptic properties, fluorescence analysis, qualitative and quantitative analysis of *Zephyranthes citrina*. Organoleptic character describes colour, odour, taste and texture. Fluorescence analysis is carried out under visible light and UV light with different chemicals and solvents. Phytochemicals are present in different solvent extracts like Hexane, Chloroform, Ethyl acetate, Acetone, Methanol and Aqueous. The plant extraction is done by using a Soxhlet apparatus. The crude extract samples were dried and the yields were calculated in grams. From the above different solvent extract, methanol extract has shown the best results in preliminary qualitative analysis. The phytochemical qualitative test for the methanol extract shows the presence of Alkaloids, Flavonoids, Phenolic compounds, Saponins, Tannin and Terpenoids. Quantitative estimation is performed to identify the constituents as alkaloids, total flavonoid, total phenolic, saponin and tannin content by standard method. The results revealed that secondary metabolites such as phenolics (6.674 ± 0.0012mg/g) and total flavonoids (1.932 ± 0.002 mg/g) were present high amounts in the extract.

Keywords: organoleptic, fluorescence, phytochemicals, soxhlet extraction, quantitative estimation

1. Introduction
Phytochemistry is the branch of chemistry concerned with plants and plant products. Medicinal plants are known to contain innumerable biologically active compounds. India is the largest producer of medicinal herbs and is appropriately called the "Botanical Garden of the World". Medicinal plants constitute the main source of new pharmaceuticals and health care products [1]. The valuable medicinal properties of different plants are due to the presence of several chemical constituents like alkaloids, tannins, phenolics, flavonoids, terpenoids, carbohydrates, glycosides, steroids, saponins, fats and oils etc., among them, some are synergistic and enhance the bioactivity of other compounds [2].
Zephyranthes citrina was described by Baker in 1882. It belongs to the family *Amaryllidaceae*. Plants of the *Amaryllidaceae* family, a small group of monocotyledonous species, include about 860 to 1100 species in eighty five genera distributed largely over tropical and sub-tropical regions [3]. The family *Amaryllidaceae* is known to contain characteristic alkaloids known as *Amaryllidaceae* alkaloids (AAs) mainly responsible for different pharmacological activities. It is a bulbous plant with green leaves dull 4mm wide. The one-inch lemon yellow flowers of this rain lily spring forth in late summer. These yellow blooms face upwards and fare open, giving them a cheerful appearance [4]. It grows luxuriantly in natural grasslands and as well as in gardens after rain fall. Since they often come into bloom after it rains, *zephyranthes* are commonly called as citron *zephyr lily* or *yellow rain lily* [5]. The main objective of the present study is to assess organoleptic character, fluorescence analysis, qualitative and quantitative estimation of *zephyranthes citrina* powder sample.

2. Materials and Methods
2.1 Collection and authentication of plant material
The plant is collected from host area of Institution and Kuchipalayam, Paramathi velur, Namakkal District. The plant is identified and authenticated in the BSI (Botanical Survey of India), Department of Botany, Agricultural University, Coimbatore. The bulbous plant material were cut into pieces, dried under shade for 15 days, coarsely powdered and stored in air tight containers for the further study.

2.2 Organoleptic study

Organoleptic is defined as being perceivable by the senses such as smell, appearance, taste, touch, odor etc. [6]. There are several ways in which to test the organoleptic properties of dried samples, including chemical or microscopic testing as well as by perceiving it directly through the senses. Organoleptic evaluation can be done by means of organs of sense and thereby define some specific characteristics of the material which can be considered as a first step towards establishment of identity and degree of purity [7]. The organoleptic investigations (condition, colour, odor, taste, texture and nature) were performed and tabulated.

2.3 Fluorescence analysis

Fluorescence is the phenomenon exhibited by various chemical components present in the plant material. In the present study, the fluorescence analysis of *zephyranthes citrina* plant powder and crude methanol extract were analyzed under visible light / daylight and UV light when treated with different chemicals and solvents [8]. The exposure of the powder and methanol crude extract to visible and UV light revealed the development of respective colors.

A small quantity of dried and finely powdered sample and crude methanol extract were treated with freshly prepared acids, alkaline solutions and different solvents. The powder sample was treated with acids viz., 50% HCl, 50% HNO_3 and 50% H_2SO_4 and acetic acid and with alkaline solutions viz., 1N alcoholic NaOH and 1N aqueous NaOH and with different solvents viz., chloroform, petroleum ether, distilled water, methanol, ammonia ferric chloride and ethyl acetate. They were subjected to fluorescence analysis in Visible light and UV light [9].

2.4 Preliminary phytochemical screening

Preparation of plant extract by soxhlet extraction

Soxhlet extraction is only required where the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. If the desired compound has a high solubility in a solvent then a simple filtration can be used to separate the compound from the insoluble substance. The advantage of this system is that instead of many portions of warm solvent being passed through the sample, just one batch of solvent is recycled. This method cannot be used for thermo labile compounds as prolonged heating may lead to degradation of compounds.

Various organic solvents were used for the extraction of bioactive compounds. The *Zephyranthes citrina sample* was dried and successfully extracted with Hexane, chloroform, Ethyl acetate, acetone, methanol and then water in a Soxhlet apparatus. The concentrated extracts were subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures [10, 20].

2.4.1 Alkaloids: Crude extract was mixed with 2 ml of Wagner's reagent. Reddish brown colored precipitate indicates the presence of alkaloids.

2.4.2 Flavonoids: To the crude extract concentrated H_2SO_4 was added. A reddish brown colouration was observed due to the presence of flavonoids.

2.4.3 Phenolic: To the crude extract add 0.5% $FeCl_3$ solution. Formation of bluish black precipitate indicates the presence of Phenolic compounds.

2.4.4 Terpenoids: 5 ml of extract was mixed with 2 ml of chloroform and 3 ml of concentrated H_2S0_4 was carefully added to form a layer. A reddish brown colouration of the inter face was formed to show positive results for the presence of terpenoids.

2.4.5 Saponin: 5 ml of extract was mixed with 20 ml of distilled water and then agitated in a graduated cylinder for 15 minutes. Formation of foam indicates the presence of saponins.

2.4.6 Tannins: 2 ml of extract was added to few drops of 1% lead acetate. A yellowish precipitate indicates the presence of tannins.

2.4.7 Carbohydrate: Fehlings A and Fehlings B reagents were mixed and few drops of extract were added. Bluish, greenish or brown coloured precipitate is obtained.

2.4.8 Steroids: 2 ml of acetic anhydride was added to 0.5ml crude extract of plant sample with 2 ml H_2SO_4. The colour changed from violet to blue or green in samples indicates the presence of steroids

2.4.9 Oil and Resin: Formation of double layers with reagents (Water).

2.4.10 Anthraquinone: 3 ml of extract was added to 3 ml of Benzene and then 5 ml NH_3 (10%) as added. Pink, Violet or Red coloration in ammonical layer is observed.

2.4.11 Coumarine: 10 % NaOH was added to the extract followed by the addition of chloroform. Yellow color was produced, shows the presence of Coumarin.

2.4.12 Glycoside: 2 ml of extract added with 2 ml of $CHCl_3$ and 2 ml CH_3COOH. Violet to Blue or Green coloration, confirmed the presence of glycosides.

2.4.13 Protein: With 1ml extract add 1ml of conc.H_2SO_4. White precipitate is appeared.

2.4.14 Anthocyanine: 2 ml of aqueous extract was added to 2 ml of 2N HCl and ammonia. The appearance of pink-red turns, blue-violet indicates the presence of anthocyanins.

2.4.15 Phlobatannins: The crude extract of plant sample was boiled with 2 % aqueous HCl. The deposition of a red precipitate was taken as evidence for the presence of phlobatannins.

2.4.16 Gardiac-glycoside: 5 ml of extract was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was mixed with 1 ml of concentrated H_2SO_4. A brown ring of the interface indicates a deoxy sugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.

2.4.17 Emodine: 2 ml of NH_4OH and 3 ml of Benzene were added to the extract. Appearance of red colour, indicates the presence of emodins.

2.4.18 Fatty acids: 0.5 ml of extract was mixed with 5 ml of ether. These extract was allowed for evaporation on filter

paper and dried the filter paper. The appearance of transparence on filter paper indicates the presence of fatty acids.

2.5 Quantitative analysis

2.5.1 Determination of alkaloids

The amount of alkaloid present is determined according to the described literature method by Harbone [21]. 5 gms. of sample was weighed, transferred into a 250 ml beaker and 200 ml of 10% CH_3COOH in ethanol was added. The solution was covered and incubated at room temperature for 2 hours. Then the solution was filtered and the filtrate was concentrated on a water bath to one quarter of the original volume. Concentrated NH_4OH was added drop wise to the extract and the precipitate was collected and washed with dilute HCl and then filtered. The residue were dried and weighed.

2.5.2 Determination of total flavonoid content

The total flavonoid content was determined with the aluminum chloride colorimetric assay [21]. 1 ml of extract or standard solution of quercetin (50, 100, 200, 400, 800 ppm) was placed in a test tube and then 4 ml of distilled water was added. The addition was finished with 0.3 ml of 5% $NaNO_2$. After 5 minutes, 0.3 ml of 10% $AlCl_3$ was added. At the sixth minute, 2 ml of 1 M NaOH put in and the total volume was made up to 10 ml using distilled water. The solution was mixed. It was then incubated for 60 minutes at room temperature. Then the absorbance against the prepared reagent, blank was determined at 510 nm using a UV-Vis spectrometer. The total flavonoid content of the extract was expressed in milligrams of Quercetin equivalents/g.

2.5.3 Determination of total phenolic content

The total phenolic content was assessed with the Folin-Ciocalteau assay [2]. Briefly, 1ml of extract was mixed with 4.5 ml of distilled water and then 1 ml of 1 M Folin-Ciocalteaus reagent was added. The mixture was vortexed for 10 minutes and was allowed to react for another 5 minutes period. Then, 2.5 ml of 7.5 % Na_2CO_3 solution was added. After incubation at room temperature for 2 hours, the absorbance of each mixture was measured at 760 nm. The same procedure was also used for the standard solution of Gallic acid (50, 100, 200, 400, 800 ppm) and a standard curve was obtained. In the results 1/10 diluted total phenolic contents were expressed in mg of Gallic acid equivalent/g.

2.5.4 Determination of saponins

The method employed for the determination of saponins was cited from reference method. 8 of gram sample were weighed and put into a flask. 100 ml of 20% aqueous ethanol solution was added. The sample was heated over a water bath at 55°C for 4 hours with incessant stirring. The mixture was filtered and the residue was re-extracted with 100 ml of 20% ethanol. The total volume of combined extracts was reduced to 35 ml over water bath at 90°C. The concentrate was transferred into a separating funnel. 20 ml of diethyl ether was added to the concentrate and shaken vigorously. The ethereal layer was discarded. This process was repeated and then 60 ml of n-butanol was added to the extract. Finally, the solution was heated on a water bath for evaporation and the samples were dried in the oven until a constant weight is obtained.

2.5.5 Determination of tannin content

5 grams of samples were extracted with 20 ml of warm water and filtered. 0.1 ml of the filtrate was added to 0.1 ml of 0.1 M ferric solution in an alkaline medium and allowed to stand for 30 minutes for color change, the absorbance was read at 760 nm and the amount of tannin was calculated from a standard calibration curve of Tannic acid. Results were expressed in mg of Tannic acid equivalent/g [21].

3. Results and Discussion

3.1 Organoleptic properties of *Zephyranthes citrina*

It is a significant parameter of powder analysis which is a technique for the qualitative detection of morphological and sensory profile of plant powder. The study revealed the characteristics, colour, odour, taste, texture and nature [22]. Organoleptic properties were studied for both the raw material and crude sample of methanolic extract [23]. The results of appearance, colour, odour, taste, texture and nature are shown in the table 1. From the analysis, it is clearly found that, colour, texture and nature are different but the other three parameters (Condition, odor and taste) are same in both the analysis.

Table 1: Organoleptic properties of *zephyranthes citrina*

S. No.	Particulars	Raw material (Plant powder)	Methanol Crude extract
1.	Condition	Dried	Dried
2.	Color	Pale yellow	Dark brown
3.	Odor	Pleasant	Pleasant
4.	Taste	Bitter	Bitter
5.	Texture	Coarse	Smooth
6.	Nature	Rough	Fine

3.2 Fluorescence analysis

Fluorescence analysis is effectively sensitive and enables the precise for the determination of components. It produces accurate result, over an adequate concentration range without several time consuming dilution steps prior to other analysis of pharmaceutical samples. The fluorescent colour is specific for each type of compound. Different plant material gives different coloration when treated with various chemicals and solvents [24, 25]. The fluorescent behavior of the powder sample and crude methanol extract of *Zephyranthes citrina* were analyzed by treated with various reagents. It is observed in visible light as well as under UV light and the observations were summarized in table 2 and 3.

Table 2: Fluorescence analysis of *Zephyranthes citrina* powder

S. No.	Treatment	Observation under Visible light	Observation under UV light
1	Powder as such	Pale Yellow	Colorless
2	Powder + Distilled water	Sandal yellow	Dark Brown
3	Powder + Petroleum ether	Light Brown	Colorless
4	Powder Chloroform	Brown	Light brown
5	Powder + Methanol	Pale Yellow	Light yellow
6	Powder + 50% HCl	Brownish Yellow	Yellowish Orange
7	Powder + 50%HNO_3	Yellowish cream	Greenish
8	Powder + 50% H_2SO_4	Dark brown	Dark brown

9	Powder + Ammonia	Sandal yellow	Light brown
10	Powder + 1N NaOH (Alcoholic)	Yellow	Light greenish
11	Powder + 1N NaOH (Aqueous)	Light brown	Light orange
12	Powder + Ferric chloride	Mustard yellow	Brownish green
13	Powder + Acetic acid	Brownish yellow	Pale brown
14	Powder + Ethyl acetate	Light yellow	Dark brown

Among the various chemical treatments, the powder sample of *zephyranthes citrina* showed the characteristic fluorescent yellow colour when treated with distilled water, methanol, 50% HCl, 50% HNO_3, Ammonia, 1 N Alcoholic NaOH, Ferric chloride, Acetic acid and Ethyl acetate under visible light. The characteristic fluorescent brown colour was observed, when treated with Distilled water, Chloroform, 50% H_2SO_4, Ammonia, Acetic acid and Ethyl acetate under UV light [9] (Table 2).

Table 3: Fluorescence analysis of crude methanol extract of *Zephyranthes citrina*

S. No.	Treatment	Observation under Visible light	Observation under UV light
1	Crude as such	Dark Brown	Yellow
2	Crude + Distilled water	Pale Yellow	Yellowish
3	Crude + Petroleum ether	Brown	Yellowish
4	Crude + Chloroform	Brown	Dark yellow
5	Crude + Methanol	Light brown	Light yellow
6	Crude + 50% HCl	Light brown	brown
7	Crude + 50% HNO_3	Yellowish orange	Greenish yellow
8	Crude + 50% H_2SO_4	Reddish brown	Dark brown
9	Crude + Ammonia	Yellowish brown	Dark brown
10	Crude + 1 N NaOH (Alcoholic)	Reddish brown	Yellow
11	Crude + 1 N NaOH (Aqueous)	Brownish yellow	Pale brown
12	Crude + Ferric chloride	Black	Dark brown
13	Crude + Acetic acid	Yellowish brown	Yellow
14	Crude + Ethyl acetate	Brown	Dark brown

Among the various chemical treatments, the crude methanol extract sample of *Zephyranthes citrina* showed the characteristic fluorescent brown colour when treated with petroleum ether, chloroform, methanol, 50% HCl, 50% H_2SO_4, Ammonia, 1 N Alcoholic NaOH, Acetic acid and Ethyl acetate under visible light. The characteristic fluorescent yellow colour was observed, when treated with Distilled water, petroleum ether, Chloroform, methanol, 50% HNO_3, 1N Alcoholic NaOH and Acetic acid under UV light[9] (table 3).

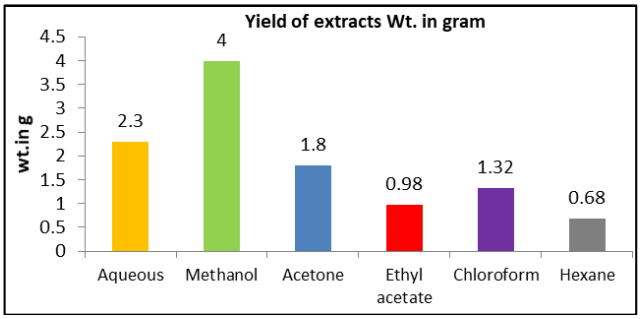

Fig 1: Yield of different solvent extracts

3.3 Quantitative yield of extract

Table 4: The yield of different solvent extracts

S. No.	Extracts	Wt. in gram
1.	Aqueous	2.30
2.	Methanol	4.00
3.	Acetone	1.80
4.	Ethyl acetate	0.98
5.	Chloroform	1.32
6.	Hexane	0.68

The crude sample of *Zephyranthes citrina* were extracted using soxhlet apparatus with above mentioned six different solvents based on the increasing order of polarity. After extraction the extract obtained were dried and weighed [26]. The yields were calculated in grams are given in table 4. The results showed that the quantitative yield of the extract. Among the six extracts, methanol extract shows the highest quantity of yield i.e., 4.00g.

3.4 Phytochemical screening

Table 5: Phytochemical screening of *Zephyranthes citrina*

S.No.	Phytoconstituents	Extracts					
		Aqueous	Methanol	Acetone	Ethyl acetate	Chloroform	Hexane
1.	Alkaloids	+	+	-	-	-	-
2.	Flavonoids	-	+	-	-	+	-
3.	Phenolic	-	+	+	-	-	-
4.	Terpenoids	-	+	+	+	+	-
5.	Saponin	+	+	-	-	-	-
6.	Tannins	-	+	-	-	-	-
7.	Carbohydrate	+	+	+	+	+	+
8.	Steroids	-	-	-	-	+	-
9.	Oil and Resin	-	-	-	-	-	+
10.	Anthraquinone	-	-	-	-	-	-

11.	Coumarine	-	-	-	-	-	-
12.	Glycoside	-	-	-	-	-	-
13.	Protein	-	-	-	-	-	-
14.	Anthocyanine	-	-	-	-	-	-
15.	Phlobatannins	-	-	-	-	-	-
16.	Gardiac-glycoside	+	-	+	-	+	-
17.	Emodine	-	-	-	-	-	-
18.	Fatty acids	-	-	-	-	-	-

The preliminary phytochemical screening of eighteen different phytochemical compounds were tested in six different solvent extracts namely hexane, chloroform, acetone, methanol, ethylacetate and water. The positive results revealed the presence of alkaloids, flavonoids, phenols, terpenoids, saponin, tannin, carbohydrate, steroids, oil and resin and gardiac-glycoside. Terpenoid and carbohydrate compounds showed the presence in ethylacetate extract. Alkaloids, saponin, carbohydrate and gardiac-glycoside compound showed the positive result in aqueous extract. Similarly two compounds present in hexane, four compounds in chloroform and three compounds in acetone. Among the six different solvent extracts, methanol extract showed the presence of maximum number (7) of compounds [27], which shows that methanol extract has highest potential activity in medicinal field.

3.5 Quantitative estimation

All the experiments were performed in triplicates and the results were expressed as mean ± standard deviation in table 6.

Table 6: Quantitative analysis of *Zephyranthes citrina*

Secondary Metabolites	Estimated quantity (mg/g)
Alkaloids	3.125 ± 0.007
Total flavonoid	1.932± 0.002
Total Phenols	6.674 ± 0.0012
Saponin	57.7375
Tannin	0.8547±0.0096

Alkaloids have a tendency to be organic and natural ingredients that have nitrogen and are also physiologically active together with sedative and analgesic roles [28]. The result showed that alkaloid content of the extract is 3.125 ± 0.007. This is the highest amount next to phenols. Flavonoids are active against free radicals, inflammation, allergies, microbes, ulcers, tumors etc [28]. Flavonoid content was determined using

Standard curve of Quercetin (y = 0.0016 + 0.2876 R^2 = 0.984) figure 2. The total flavonoid content of the extract is 1.932± 0.002 mg/g.

Phenolic compounds are most widespread molecules among plant secondary metabolites, are known to act as natural antioxidants. Phenolic compounds are most important plant constituents because of their hydroxyl groups confer radical scavenging ability [29]. The total phenolic content was calculated by gallic acid equivalent from the standard calibration curve of gallic acid (y = 0.0012 + 0.0822 R^2 = 0.9992) figure 3. The result indicates that the total phenolic content of the extract is high compared to all the other analysis. The amount of total phenolic content was 6.674 ± 0.0012 mg/g.

Saponin has numerous attention due to their biological activities that including hepatoprotective, antitumour, antimicrobial and anti-inflammatory activities [30]. Based on the result obtained, saponin content is 57.7375 mg/g. Tannin content has attracted a lot of attention in recent years because of their multifunctional properties to human health [30]. Tannin content of the extract was determined using standard curve equation derived from standard curve of tannic acid(y = 0.0011 + 0.1225 R^2 = 0.9935) figure 4. The tannin content of the extract was 0.8547±0.0096 mg/g.

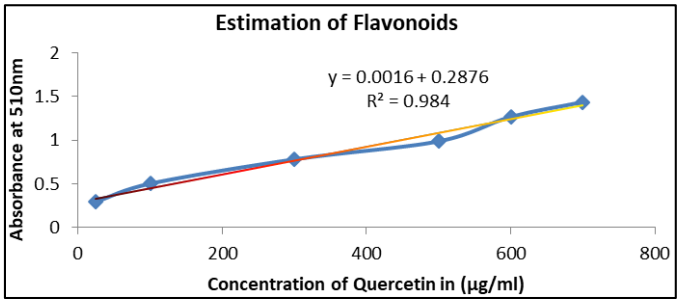

Fig 2: Standard curve of Quercetin

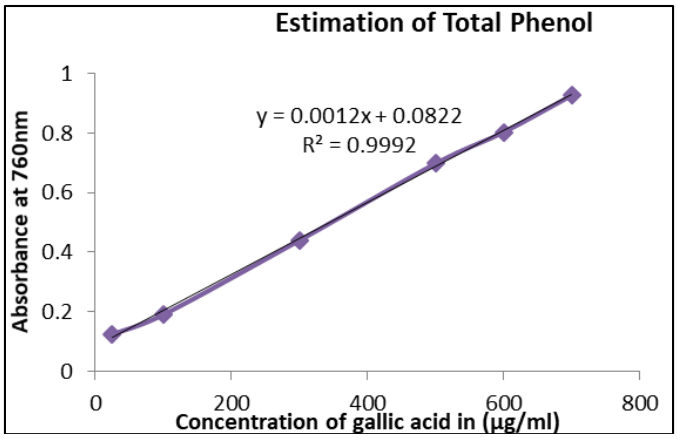

Fig 3: Standard curve of gallic acid

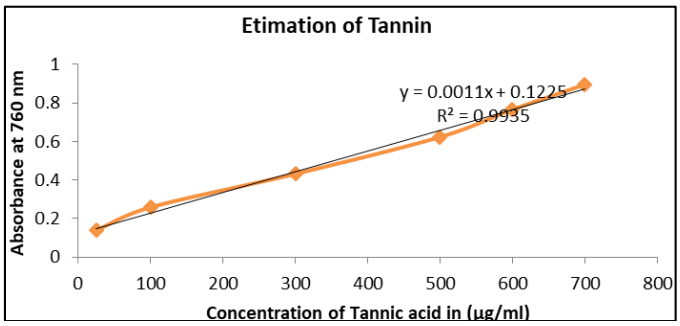

Fig 4: Standard curve of tannic acid

4. Conclusion

Based on the results obtained in the present investigation, the results of organoleptic properties showed that the taste and odour of a plant sample may influence its differential in medicinal use. The fluorescent analysis of the sample plays an important role in the determination of quality and purity of the sample. The result revealed that, the sample when viewed under UV light and visible light showed different colours at different wavelength. This is due to the presence of different

chemical constituents in the extract. The various phytochemical qualitative tests for the different solvent extracts confirm the presence of different phytoconstituents. Among these extracts methanol extract shows the presence of alkaloids, flavonoids, phenolic compounds, saponins, tannin and terpenoids. The quantitative estimation of the present study showed that the bulb extract of *Zephyranthes citrina* contains various bioactive molecules in different quantities.

5. References

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