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Pharmacological benefits of *Gerbera jamesonii* Adlam flower: Qualitative and quantitative analysis of the extract

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Abstract

This study aimed to investigate the phytochemical potential of the ethanolic extract of *Gerbera jamesonii* Adlam flowers and to quantify the total phenolic, flavonoid, and alkaloid contents in this extract. The total phenolic content was measured using the Folin-Ciocalteu method and expressed as gallic acid equivalents. The total flavonoid content was determined by the aluminum chloride colorimetric method and expressed as rutin equivalents. The total alkaloid content was measured based on the reaction between alkaloids and bromocresol green (BCG) and expressed as atropine equivalents. Qualitative phytochemical analysis of ethanolic extract of *G. jamesonii* revealed the presence of various pharmacologically active compounds, including alkaloids, carbohydrates, reducing sugars, saponins, phytosterols, phenolic compounds, and flavonoids. The total phenolic, flavonoid, and alkaloid contents were found to be 39.8 mg GAE/g, 25.39 mg RE/g, and 10 mg AE/g, respectively. Based on the qualitative and quantitative analysis of key phytochemicals, such as flavonoids, alkaloids, and phenols, this plant appears to have significant medicinal potential and could be developed as a therapeutic agent in the future.

1. Introduction

Herbal medicines are natural substances derived from plants and are employed in traditional local or regional medicine to treat various ailments. These substances are complex mixtures of organic compounds that can be derived from any raw or processed part of a plant. When comparing herbal remedies to conventional forms of medicine, it can be observed that herbal remedies are relatively more cost-effective. Herbal remedies are often perceived as having a higher efficacy compared to conventional pharmaceuticals in the treatment of certain ailments. Multiple segments within a plant organism may contain diverse active chemical compounds. Throughout history, flowers have been utilized by human beings as medicinal remedies in various regions across the globe. The utilization of flowers and their extracts in herbal therapy is employed for the treatment of a diverse range of ailments. The Asteraceae family, consisting of around 1600 genera and 2500 species worldwide, is recognized as one of the largest families within the realm of flowering plants. Over the course of history, there has been extensive documentation highlighting the significant contribution of Asteraceae family members to traditional medicine, owing to their diverse range of beneficial applications. (Pelkonen *et al.,* 2014; Rolnik *et al.,* 2021).

The *G. jamesonii* flower, belonging to the Asteraceae family, exhibits a diverse array of biological effects, encompassing anticancer, antiproliferative, antioxidant, anti-inflammatory, antiangiogenic, and cholesterol-reducing properties. (Negm El-Dein *et al.,* 2022). Since there has not been much research done on *G. jamesonii,* it would be quite interesting to carry out biological and phytochemical analysis of the plant cultivated in India to gain insight into some of its phytochemical components and assess its pharmacological potential.

Figure 1: *G. jamesonii* **flower.**

1.1 Plant description: *G. jamesonii*

The flowering plant species in the Gerbera genus, called *G. jamesonii*, belongs to the vast Asteraceae (or Compositae) family's basal

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Mutisieae tribe. The *Barberton daisy*, also referred to as the *Transvaal daisy*, is a native of South Eastern Africa. It was the first *Gerbera* species that received a scientific description and was first published in 1889 by J. D. Hooker in Curtis' Botanical Magazine. The tufted perennial herb, *G. jamesonii* progresses up to 75 cm in height and possesses bare flowering scapes. Petiolate, strongly undulating or lobed leaves are organized in a rosette and range in size from 15 to 42 cm (up to 68 cm). It has deeply lobed leaves that are covered in silky hairs that grow from a crown. The plant produces magnificent blossoms (capitula) with ray florets that are typically orange-red but can occasionally be yellow, orange, white, or pink. It has asexual reproduction and blooms that spread from September to December., (Bhat *et al.,* 2013; Akhtar *et al.,* 2020; Manning and John, 2019).

Table 1: Taxonomical classification of *G. Jamesonii*

Kingdom	Plantae
Phylum	Tracheophytes
Subphylum	Angiosperms
Class	Asterids
Order	Asterales
Family	Asteraceae
Genus	Gerbera
Species	Jamesonii

Source: Integrated Taxonomic Information System

Table 2: Scientific research reported for G. jamesonii flower

S. No	Biological activity	Plant part	Extract	Model/cell lines	References
1	Anticancer activity	Flower	Methanolic	549-Adenocarcinoma human alveolar basal epithelial cell line	Negm El-Dein *et al.,* 2022
2	Anti-proliferative activity	Flower	Methanolic	Lines of human cancerous cells Both the HCT-116 and the MCF-7	Negm El-Dein *et al.,* 2022
3	Anti-oxidant activity	Flower	Methanolic	DPPH Free radical scavenging activity	Negm El-Dein *et al.,* 2022
4	Anti-inflammatory activity	Flower	Methanolic	Anti-inflammatory assay is a method for stabilising the membrane of human red blood cells (HRBC).	Negm El-Dein *et al.,* 2022
5	Cholesterol reducing potential	Flower	Methanolic	Cholesterol reduction assay	Negm El-Dein *et al.,* 2022
6.	Antiangiogenic activity	Flower	Aqueous	Expansion of existing blood arteries into the formation of new ones	Satyarum *et al.,* 2019

2. Materials and Methods

2.1 Collection and identification of plant material

Fully mature flowers of *G. jamesonii* were collected in February 2023 from the Gudimalkapur neighborhood in Hyderabad. The flowers were identified and authenticated with Voucher No. OUAS-102 by Dr. A. Vijaya Bhasker Reddy, Assistant Professor, Department of Botany, Osmania University, Hyderabad, Telangana, India. A voucher specimen has been deposited at the Department of Botany, Osmania University for future reference.

2.2 Preparation of extract

The collected *G. jamesonii* flowers were thoroughly washed with distilled water to remove impurities, dried at room temperature, and coarsely powdered using an electric grinder. A specific quantity of this powdered material (*e.g.,* 200 g) was placed in a filter paper thimble and inserted into the main chamber of a Soxhlet apparatus. Ethanol (95%) was used as the solvent, with a sufficient volume (*e.g.,* 500 ml) poured into a round-bottom flask. The Soxhlet apparatus, including the round-bottom flask, condenser, and thimble

holder, was assembled. The extraction process was initiated by heating the ethanol, causing it to evaporate, condense, and drip onto the powdered flower material. The ethanol dissolved the phytochemicals and was siphoned back into the flask, repeating the cycle for 6-8 hours for thorough extraction. Post-extraction, the ethanol extract was concentrated by evaporating the solvent with a rotary evaporator at a controlled temperature (*e.g.,* 40°C) to prevent photochemical degradation. The concentrated extract was dried to remove any remaining solvent, yielding the ethanolic extract of *G. jamesonii*. The resulting extract was stored in an airtight container and subjected to phytochemical analysis to determine its composition (Chaisawang wong and Gritsanapan, 2009).

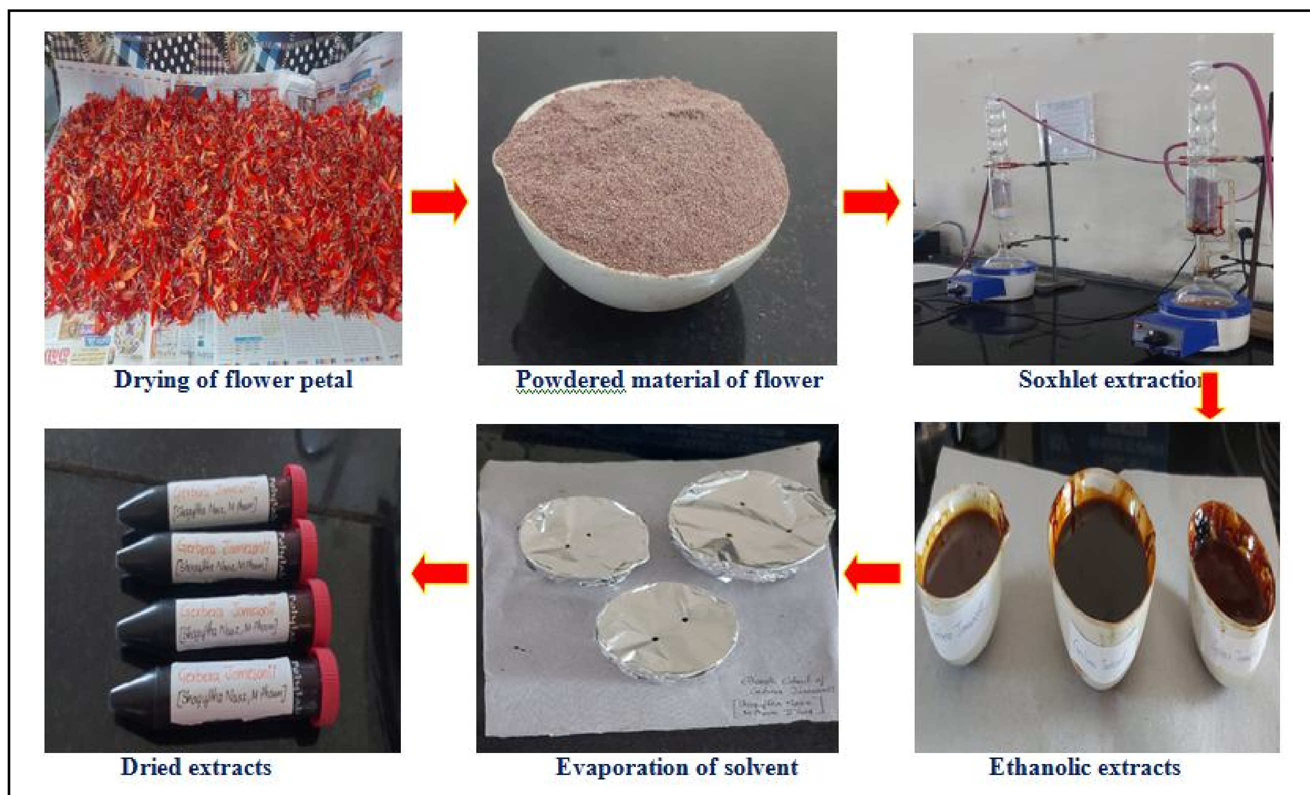

Figure 2: Preparation of *G. jamesonii* flower extract.

2.3 Qualitative analysis (Khandelwal, 2006; Chaudhari *et al.*, 2018)

2.3.1 Test for alkaloids

2.3.1.1 Mayer's test

A volume of 2-3 ml was extracted from the filtrate, and subsequently, 1-2 droplets of Mayer's reagent were introduced. An opaque, pale-coloured solid was visually detected.

2.3.1.2 Dragendroff's test

2-3 ml of the filtrate was extracted and 1-2 drops of Dragendorff's reagent were added. The formation of a reddish-brown precipitate was observed.

2.3.2 Test for carbohydrates

2.3.2.1 Resorcinol test

A 2 ml aliquot of an aqueous extract was mixed with a small amount of resorcinol crystals. An equal volume of hydrochloric acid was then added, and the mixture was heated. The resulting color change indicates the presence of ketones.

2.3.2.2 Starch test

A volume of 2 ml of extract was taken and combined with 5 millilitres of a 5% solution of potassium hydroxide. This resulted in the manifestation of a canary colouration.

2.3.3 Test for reducing sugars

2.3.3.1 Benedict's test

0.5 millilitres of extract were combined with benedict's reagent and then boiled. This produced a color spectrum ranging from green to yellow to red.

2.3.3.2 Fehling's test

To initiate the experiment, 1 ml of the substance was extracted and fehling A and B solutions were added. The mixture was then boiled in a water bath, resulting in the formation of a crimson precipitate.

2.3.4 Test for saponin

2.3.4.1 Froth test

For optimal outcomes, the extract was diluted with water up to 20 ml in a graduated tube and vigorously shaken for 15 min. The resulting foam measured 1 cm in height.

2.3.4.2 Foam test

2 ml of extract was combined with 1 millilitre of water, and then the mixture was vigorously shaken. It typically took around 10 min for foam to form.

2.3.5 Test for phytosterols

2.3.5.1 Salkowski's test

A few drops of strong hydrochloric acid was added to the extract and dissolved it in 5 ml of chloroform. The result is a brownish rim.

2.3.6 Test for phenolic compounds

2.3.6.1 Ferric chloride test

2-3 millilitres of extract were taken and 3-4 drops of ferric chloride solution were added. This resulted in a hue that combined blue and black.

2.3.6.2 Lead acetate

A volume of 2 ml of the extract was taken and combined with 3 ml of a solution containing 10% lead acetate. The observed outcome was the generation of a voluminous white solid.

2.3.7 Test for flavonoids

2.3.7.1 Lead acetate test

2 ml of the extract was measured out, and a small quantity of lead acetate was introduced using a few droplets. The resulting precipitate exhibited a golden hue.

2.3.7.2 Ferric chloride test

2 ml of extract was used, and a small quantity of Ferric chloride was added using a few droplets. This resulted in a vivid green hue.

2.3.8 Test for proteins and amino acids

2.3.8.1 Millon's test

A small quantity of the extract was dispensed, and then 2 ml of Millon's reagent was added. Upon the addition of a certain substance, a white precipitate formed. Upon heating, the precipitate underwent a color change, transitioning to a brick-red hue.

2.3.8.2 Biuret test

Take 2 ml of the extract and mix it with a small quantity of 2% copper sulphate solution, along with 1 ml of ethanol. Then, introduce an excess of solid potassium hydroxide pellets. This addition caused the extracted layer to acquire a pink hue.

2.3.8.3 Ninhydrin test

2 ml of the extract was placed into a vessel, and ninhydrin reagent was added. The mixture was boiled, resulting in a blue hue.

2.3.9 Test for cardiac glycosides

2.3.9.1 Keller-Killiani test

To initiate the experiment, extract a small amount and mix it with two millilitres of glacial acetic acid. Then, add a few drops of ferric chloride and one millilitre of concentrated sulphuric acid. The presence of deoxy sugar properties in cardenolides leads to the formation of a brown ring. Below the brown ring, a violet ring may appear, and a green ring can be observed in the acetic acid layer.

2.3.10 Test for fixed oils and fats

2.3.10.1 Spot test

Position the extract within the confines of the filter paper. An oil smear has been seen.

2.4 Quantitative analysis

2.4.1 Total phenolic content

To determine the total phenolic content using the Folin-Ciocalteu method, gallic acid was used as the standard. Six different concentrations of gallic acid standard solutions were prepared at 1, 5, 10, 20, 40, and 50 µg/ml. For the sample preparation, 100 mg of the sample extract was accurately weighed and dissolved in 100 ml of triple-distilled water (TDW) to create a 1 mg/ml solution. In the assay procedure, 1 ml of the sample extract solution was transferred into a test tube. To this, 0.5 ml of 2N Folin-Ciocalteu reagent was added, and the mixture was allowed to stand for 5 min at room temperature. Subsequently, 1.5 ml of 20% sodium bicarbonate solution was added, and the total volume was brought up to 8 ml with TDW. The mixture was then vigorously shaken to ensure thorough mixing. The reaction mixture was left to stand for 2 h at room temperature to allow for color development. After the incubation period, the absorbance of the mixture was measured at 765 nanometers using a spectrophotometer, with TDW serving as the blank. To quantify the phenolic content, a calibration curve was created by plotting the absorbance values of the gallic acid standard solutions against their respective concentrations. Using this calibration curve, the concentration of phenolic compounds in the sample extract was determined and expressed as milligrams of gallic acid equivalents per gram of extract (mg GAE/g) (Baliyan *et al.,* 2022; Siddique *et al.,* 2010).

2.4.2 Total flavonoid content

The principle behind this procedure is the formation of a complex between flavonoids and aluminum, which exhibits maximum absorptivity at a wavelength of 415 nm. Rutin was used as the standard for the assay. Six standard solutions of rutin were prepared at concentrations of 1, 5, 10, 20, 40, and 50 µg/ml. For the sample preparation, 100 mg of the sample extract was accurately weighed and dissolved in 100 ml of triple-distilled water (TDW). To conduct the assay, 1 ml of the sample extract solution was transferred to a test tube. To this solution, 0.3 ml of 5% sodium nitrite was added and allowed to react for 5 min. After this incubation period, 0.3 ml of 10% aluminum chloride was added to the mixture and allowed to stand for 10 min. Subsequently, 2 ml of 1 M sodium hydroxide was added to the reaction mixture. The mixture was then diluted by adding 3.3 ml of distilled water and mixed thoroughly to ensure homogeneity. After 40 min, the absorbance of the mixture was measured at 415 nm using a spectrophotometer. This procedure allowed for the quantification of the flavonoid content in the sample extract by comparing the absorbance values to those obtained from the standard rutin solutions. The results were expressed as milligrams of rutin equivalents per gram of extract (mg RE/g) (Baliyan *et al.,* 2022; Siddique *et al.,* 2010).

2.4.3 Total alkaloid content

Atropine was used as the standard for this assay, with six standard solutions prepared at concentrations of 20, 40, 60, 80, and 100 µg/

ml. To prepare the sample, accurately weigh 100 mg of the sample extract and dissolve it in 100 ml of triple-distilled water (TDW). Transfer 1 ml of this plant extract into a separatory funnel and wash it with 10 ml of chloroform to remove non-alkaloid impurities. To neutralize the solution, add a small amount of 0.1 N NaOH. Next, add 5 ml of bromocresol green (BCG) solution and 5 ml of phosphate buffer to the separatory funnel. Vigorously shake the mixture to ensure thorough mixing and allow the complex between the alkaloids and BCG to form. After shaking, separate the chloroform layer, which contains the alkaloid-BCG complex. Transfer this chloroform extract into a 10 ml volumetric flask and dilute it with additional chloroform to make up the final volume. The absorbance of the complex in chloroform was measured at 470 nm against blank prepared as above but without atropine (Baliyan *et al.,* 2022; Siddique *et al.,* 2010).

3. Results

3.1 Qualitative analysis of ethanolic extract of *G. jamesonii*

The phytochemical analysis of ethanolic extract of *G. jamesonii* showed the presence of alkaloids, carbohydrates, reducing sugars, saponin, phytosterols, phenolic compounds, and flavonoids

Table 3: Phytochemical screening of ethanolic extract of *G. jamesonii*

S. No.	Phytochemical test	*G. jamesonii* extract
1.	Alkaloids	Present
2.	Carbohydrates	Present
3.	Reducing sugars	Present
4.	Saponin	Absent
5.	Phytosterols	Highly present
6.	Phenolic compounds	Present
8.	Flavonoids	Present
9.	Proteins and amino acids	Absent
10.	Cardiac glycosides	Absent

3.2 Quantitative analysis of ethanolic extract of *G. jamesonii*

3.2.1 Total phenolic content

The total phenolic content in the ethanolic extract of *G. jamesonii* was quantified using the regression equation derived from the gallic acid calibration curve. The analysis revealed a total phenolic content of 39.8 mg gallic acid equivalents (GAE) per gram of extract.

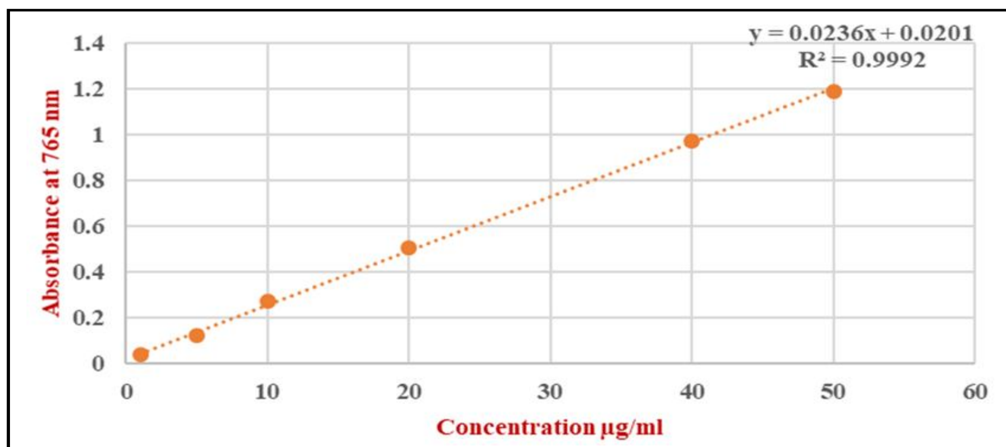

Figure 3: Standard calibration curve of gallic acid.

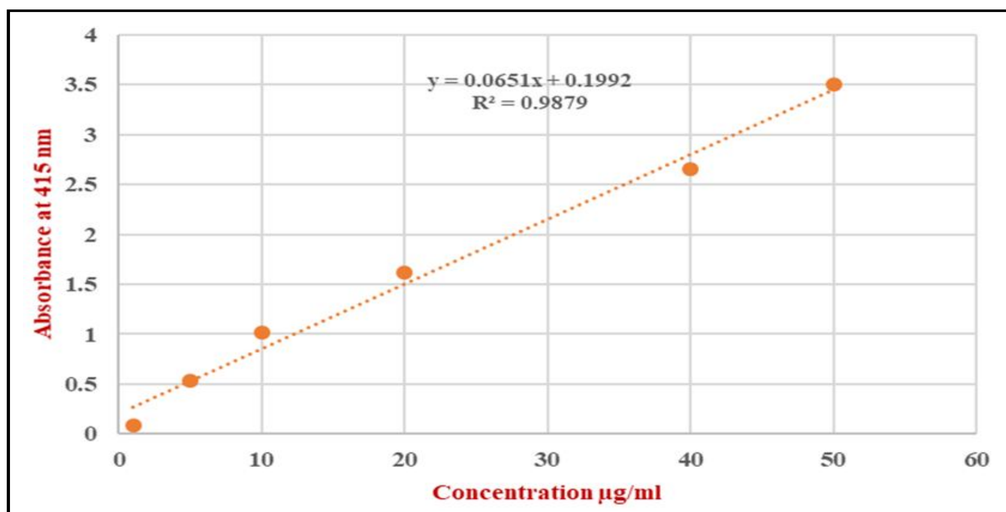

Figure 4: Standard calibration curve of rutin.

3.2.2 Total flavonoid content

The total flavonoid content in the ethanolic extract of *G. jamesonii* was assessed using the regression equation derived from the Rutin calibration curve. The analysis revealed a total flavonoid content of 25.39 mg rutin equivalents (RT) per gram of extract.

3.2.3 Total alkaloidal content:

The total alkaloidal content in the ethanolic extract of *G. jamesonii* was evaluated using the regression equation obtained from the calibration curve. The analysis yielded a total alkaloidal content of 10 mg atropine equivalents (AT) per gram of extract.

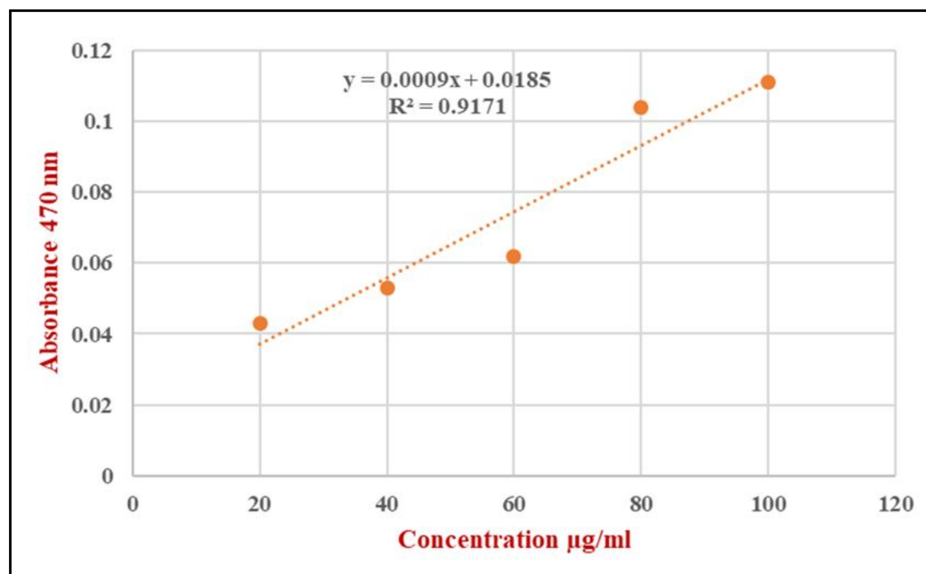

Figure 5: Standard calibration curve of atropine.

4. Discussion

Plants have long been recognized globally as a vital reservoir of raw materials for the synthesis of traditional and contemporary medicines. Research into the medicinal properties of plant species plays a crucial role in validating their therapeutic potential, offering a promising avenue for the development of cost-effective and efficacious treatments utilizing readily available natural resources. Across the world, an estimated 50,000 plant species possess therapeutic attributes, underscoring the immense diversity of potential sources for medicinal compounds. In traditional medicinal systems such as Ayurveda, Chinese medicine, and Unani medicine, herbs, shrubs, and trees are extensively utilized either in their crude form or as components of formulated medicines. Many important drugs, including aspirin, digoxin, morphine, and quinine, are derived from plants and are employed in the treatment of various disorders. Given this wealth of medicinal potential, both qualitative and quantitative analyses of the phytochemical constituents of plants like *G. jamesonii* are undertaken to identify active compounds. Such analyses provide valuable insights into the therapeutic properties of plant extracts, paving the way for the development of novel pharmaceutical agents derived from natural sources.

The qualitative phytochemical analysis of the ethanolic extract of *G. jamesonii* revealed the presence of several significant bioactive compounds. The analysis identified alkaloids, which are known for their pharmacological effects, including analgesic and anti-inflammatory properties. Carbohydrates and reducing sugars were also detected, indicating the presence of essential energy sources and metabolic intermediates. The presence of saponins was noted, compounds known for their potential benefits such as immune-boosting and cholesterol-lowering effects. Phytosterols were identified, which are plant-derived sterols that can contribute to lowering cholesterol levels and improving cardiovascular health. Additionally, phenolic compounds were detected, which are known for their antioxidant properties and their role in preventing oxidative stress-related diseases. Finally, flavonoids were found, which are known for their wide range of biological activities, including anti-inflammatory, anticancer, and antioxidant effects. This comprehensive profile of phytochemicals suggests that the ethanolic extract of *Gerbera jamesonii* has significant potential as a source of therapeutic agents.

Quantitative analysis determined the total phenolic content using the gallic acid calibration curve (39.8 mg GAE/g), total flavonoid content using the rutin calibration curve (25.39 mg RT/g), and total alkaloid content using the atropine calibration curve (10 mg AT/g). These results indicate high levels of phenolic and flavonoid compounds in EEGJ.

5. Conclusion

The findings of this study underscore the rich phytochemical composition of *G. jamesonii*, known for its diverse medicinal properties and pharmacological effects. The high levels of phenolic and flavonoid compounds observed in the extract suggest its significant bioactivity. Moving forward, future research avenues could focus on elucidating the specific mechanisms of action of these bioactive compounds and exploring their potential therapeutic applications. Additionally, investigating the synergistic effects of these compounds in combination with conventional medicines or other natural extracts could enhance their efficacy and expand their clinical utility. Continued research into *G. jamesonii* and similar plant species holds promise for the development of innovative

pharmaceuticals and complementary therapies to address various health conditions.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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