Estimation of phytochemicals, biological activities, and formulation of cream from the extract of *Gaultheria fragrantissima* Wall

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Abstract: *Gaultheria fragrantissima* Wall. is a medicinal plant widely used in traditional systems of medicine due to its potent therapeutic effects as an anti-inflammatory and analgesic agent. In the present study, the methanol extract and an essential volatile oil of *G. fragrantissima* were employed as the active ingredient for the formulation of vanishing cream, and their physicochemical and biological properties were studied. The methanol extract and volatile oil were analyzed for extractive values, ash values, and loss on drying, and total phenolic and total flavonoid contents. Antioxidant and cytotoxic potentials were assessed using DPPH and MTT assays, respectively. The quantitative phytochemical analysis revealed that the methanol extract contained the highest total phenolic content (111.94 ± 1.36 mgGAE/g) and flavonoid content (50.02 ± 0.89 mgQE/g). Furthermore, the extract possessed strong antioxidant activity, as evidenced by the low IC_{50} value of 59.80 ± 0.58 µg/mL. In comparison to essential oil, methanol extract demonstrated notable cytotoxic potential with CC_{50} values of 41.68 ± 0.39 µg/mL in breast (MCF-7) and 57.09 ± 1.26 µg/mL in lung (A549) cancer cell lines. Six batches of the formulated cream met the required standards for pH, homogeneity, and emollience and remained stable throughout the stability testing period. The physicochemical parameters are within the range given in the Ayurvedic Pharmacopoeia of India. The prepared creams were slightly acidic in nature, but they had excellent homogeneity and emollience. The creams retained their physical attributes throughout the 28-day testing period. This study highlights the potential of G. fragrantissima in the formulation of cream with potential biomedical applications.

Keywords: Analgesic; Cream formulation; Cytotoxicity; DPPH; MTT.

Introduction

Natural products have been extensively used in healthcare traditionally and continue to play a significant role in modern medicine despite major advances in synthetic chemistry and biotechnology due to their therapeutic and preventive properties with no or relatively small side effects[1]. One of the main sources of materials utilized in the pharmaceutical and cosmetics industry is botanicals [2]. They are used for multiple purposes, including for inflammatory skin conditions like psoriasis and skin care, antioxidant, antimicrobial, hydrating, repairing, cleansing, or whitening [3]. Cosmetics are typically applied directly to the outer surfaces of the human body to serve four purposes: (1) to keep the body in excellent shape; (2) to alter appearance; (3) to provide protection; and (4) to address body odour [4]. Pharmaceutical companies are increasingly interested in plant-based cosmetics, driven by a growing demand for natural products due to their minimal toxic side effects [5]. Herbal cosmetics are free from harmful synthetic chemicals, rich in nutrients and antioxidants, and ensure no allergic reaction or irritation to the skin [6].

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Received: 23 Feb, 2026; Received in revised form: 21 Mar, 2026; Accepted: 26 Mar, 2026.
Doi: https://doi.org/10.3126/sw.v19i19.95619

Creams are semi-solid emulsions, either oil-in-water (O/W) or water-in-oil (W/O), designed for topical application to skin or mucous membranes with improved skin penetration and patient compliance for a variety of therapeutic and cosmetic uses[5,7]. There is a wide variety of plants, which can be used to produce cosmetic products from the extract obtained from their parts: leaves, flowers, roots, seeds, and stems [8]. The therapeutic and beautification effects of plant-based cosmetics are facilitated by a variety of phytoconstituents, including flavonoids, alkaloids, tannins, saponins, quinines, terpenoids, glycosides, polyphenols, and fats and oils1. The ongoing global resource limitations and environmental crisis demand a sustainable solution for the cosmetic sector in order to satisfy customers' growing need for healthy, natural, and eco-friendly products. Plant source is one of the main alternatives to address this issue in the cosmetic field [8].

Cancer remains one of the most serious global health concerns and the leading cause of mass mortality, characterized by uncontrolled cell proliferation, resistance to apoptosis, and the ability to invade and metastasize. Lung cancer is the leading cause of cancer-related mortality globally, accounting for approximately 1.8 million deaths[9,10], while breast cancer is the most commonly diagnosed cancer among women[11]. Conventional cancer treatments are widely used in the form of surgery, chemotherapy, and radiotherapy, while their long-term effectiveness is limited due to extreme toxicity, adverse side effects, and development of drug resistance, calling for the need for safer and more effective therapeutic alternatives, again redirecting towards natural products[10]. Medicinal plants are potent sources of anticancer agents because of their ability to modulate oxidative stress, inflammation, apoptosis, and tumor progression. At present, nearly half of the currently used anticancer drugs are derived from natural products or their analogues[12,13,14.]

Gaultheria fragrantissima is a tiny, robust, woody, evergreen perennial shrub that is typically found in the hilly areas of North Eastern India, including Indo-Nepal and Indo-Bhutan regions, at elevations between 1800 and 2500 m, as well as in the hills of Tamil Nadu and Kerala, India, at elevations greater than 1500 m15. In Nepal, it is commonly known as Dhasingre, and its essential oil is one of the most exported oils from Nepal. It has been reported that Gaultheria species are used to treat inflammatory disorders, rheumatoid disorders, swelling and pain, chronic tracheitis, and chronic prostatitis. The presence of methyl salicylate is mainly responsible for the analgesic and anti-inflammatory effects of Gaultheria[16].

Figure 1: Fruits of *Gaultheria fragrantissima.*

Many studies have elucidated that the species has medicinal and cosmetic uses: as in aromatherapy, fragrance-based industries[17], against various skin conditions for its antioxidant, antibacterial, anti-inflammatory, analgesic properties[18,19], against rheumatoid arthritis, influenza, cough, asthma, and pain of various causes, as well as promote wound healing[20]. Furthermore, studies since the 1940s have shown the anticancer potential of Gaultheria species, with both volatile and non-volatile constituents comprising diterpenoids and dilactones, exhibiting promising cytotoxic activity against various cancer cell lines[21]. Moreover, this plant species has been used extensively in the fragrance industry, aromatherapy, and food preservation. In this study, G. fragrantissima leaves' volatile and non-volatile phytochemicals are extracted by following hydrodistillation and maceration, and their total phenolic content (TPC), total flavonoid content (TFC), antioxidant, and cytotoxic potential are analysed. Further, vanishing cream formulations were prepared using the plant extracts and assessed utilizing initial quality control tests.

Material and methods

Plant material collection and authentication

Fresh Leaves of G. fragrantissima were collected from

Telkot forest, near Nagarkot, at an altitude of 2000 m, in April-May 2023. The National Herbarium and Plant Laboratories, Godawari, Lalitpur, identified the sample species.

Chemicals and Equipment

Reagents were sourced from various companies. Gallic Acid, Folin-Ciocalteu, from Loba Chemie. Quercetin, and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) from Merck Life Science, Trypsin-EDTA, Fetal bovine serum, Actimycotic-Antibiotic, and Phosphate buffer saline from Gibco Laboratories, Dulbecco's Modified Eagle Medium (DMEM), and Doxorubicin from Sigma-Aldrich. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT reagents from Sisco Research Laboratories. Equipment used was Clevenger's apparatus, a double beam UV-visible spectrophotometer (Shimadzu), a muffle furnace (Tempo Instruments), a moisture balance (Spectra lab), a hot air oven (Thermolab), and a water bath (Equitron). pH meter (Spectra lab), real-time stability chamber (Osworld), and accelerated stability chamber (Thermolab).

Phytochemical extraction

Freshly collected leaves of G. fragrantissima were washed properly with tap and distilled water to remove the dust particles. The leaves were then chopped into fine pieces and dried in the shade for 10 days. The shade-dried leaves were ground into fine powder using an electric grinder and stored in a clean airtight polythene bag. A finely powdered sample was subjected to extraction by cold maceration utilizing methanol solvent[22].

Extraction of essential oils

The Clevenger apparatus was used following the hydrodistillation method[23].

Identification of volatile oils

Identification of the volatile oils was carried out according to the Ayurvedic Pharmacopoeia of India[24].

Organoleptic characteristics

The volatile oil was evaluated for its color, odour, and taste at room temperature. The color of the oil was observed against a plain white background, while the taste and odor were assessed to check the characteristic aromatic and pungent properties as per the mentioned guidelines in the Ayurvedic pharmacopoeia of India.

Identification of phenolic compounds

In this method, approximately 2 mL of the volatile oil was first taken in a clean test tube. To this solution, one drop of ferric chloride solution was added. Then the mixture was gently shaken, and color development was noticed.

Physicochemical analysis of leaf powder

Alcohol soluble extractive value

Approximately 5 g of leaf powder was weighed into a 250 mL conical flask with a stopper, and 100 mL of ethanol was added. The mixture was left overnight with occasional shaking and then filtered. Around 25 mL of the filtrate was pipetted out and evaporated to dryness in a weighed shallow flat-bottomed petri dish on a water bath maintained at 80 °C. Thus, the obtained residue was dried at 105 °C to a constant weight in a hot air oven[25].

Water-soluble extractive value

The water-soluble extractive value of leaf powder was analyzed by adopting the given protocol [26] with slight modifications. In this method, approximately 5 g of leaf powder was weighed into a 250 mL conical flask with a stopper, and 100 mL of water was added. The flask was shaken occasionally and left to stand overnight. The mixture was filtered. Around 25 mL of filtrate was pipetted out and evaporated to dryness in a weighted shallow flat-bottomed petri dish on a water bath maintained at 80 °C. Thus, the obtained residue was dried at 105 °C to a constant weight in a hot air oven.

Total ash determination

Total ash of the sample was determined by using the standard protocol[27]. In brief, 4 g of leaf powder was taken in a previously ignited and tarred silica dish. The powder was spread evenly in the dish and ignited in a muffle furnace at 600 °C until it was white, indicating the absence of carbon. The dish was kept in a desiccator until it was

cooled and then weighed. The percentage of total ash was calculated by using the formula:

$$Total\ ash = \frac{Wt.of\ ash\ obtained}{Wt.of\ sample\ taken} \times 100\% \quad \ldots\ldots\ldots (1)$$

Acid-insoluble ash determination

A total of 45 mL of 1:5 (v/v) hydrochloric acid was added to the dish containing the total ash in three portions (15 mL each time), and was boiled gently for 5 minutes and filtered. The insoluble matter was collected on ashless filter paper and washed with distilled water until the residues were free from acid. The filter paper containing the insoluble matter was transferred to the original dish, dried, and ignited to constant weight. The acid-insoluble ash was calculated after cooling and weighing the dish [27].

Moisture content

Two grams of the leaf powder were analyzed in a moisture balance, and the moisture content was noted[28].

Quantitative analysis

Estimation of total phenolic content

Total phenolic content (TPC) of the plant extract was estimated by the Folin-Ciocalteu colorimetric method[29].

Estimation of total flavonoid content

Total flavonoid content (TFC) of the plant extract was estimated by the Aluminum chloride colorimetric method[30,31].

Investigation of antioxidant activity

Antioxidant activity of the plant extract and the essential oil of G. fragrantissima was determined by using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay[32]. As a standard reference antioxidant, quercetin was taken. The % scavenging was calculated by using the formula:

$$Scavenge\ (\%) = \frac{(A_o - A_s)}{A_0} \times 100\% \quad \ldots\ldots\ldots\ldots (2)$$

Where, 'Ao' = Control Absorbance and 'As' = Sample absorbance.

The IC_{50} values (50 % inhibitory concentration) of the samples were calculated by using GraphPad Prism software.

Cytotoxic potential

The cytotoxic potential of essential oil and methanolic extracts of G. fragrantissima was determined by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) MTT assay[33]. Human cancer cell lines: lung (A549) and breast (MCF-7) cancer cell lines, were treated with variable concentrations of essential oil and methanol extract in a triplicate manner. The standard anticancer drug doxorubicin was taken as a reference drug. The treated cells of about 1×104 cells per well were incubated for 48 h in 5 % CO_2 at 37 °C. After 48 h of incubation, the supernatant was aspirated, 100 µl of DMEM and 20 µl of 5 mg/mL MTT reagent were introduced, and kept for 4 h for the formation of formazone. The formazone crystals were solubilized by using 100 % DMSO, and the optical density was recorded at 570 nm. The percentage cytotoxicity of the samples was determined by using the equation.

$$Cytotoxicity\ (\%) = \frac{A_0 - A_S}{A_0} \times 100\% \ldots\ldots (3)$$

Where, 'Ao' = Control Absorbance and 'As' = Sample absorbance

50 % cytotoxicity concentration (CC50) of each sample was computed using GraphPad Prism 10 software.

Preparation of cream

A sample oil-in-water (o/w) cream containing G. fragrantissima extract was formulated. The aqueous and oil phases were taken into beakers separately and heated to 75 ± 5°C on a water bath. The oil phase consisted of cetostearyl alcohol, stearic acid, and white soft paraffin, while the aqueous phase consisted of sodium carbonate, potassium hydroxide, ethanol, glycerin, and water. Slow addition of the oil phase to the water phase was done with constant stirring until the mixture cooled down to room temperature34. The purposes of the different ingredients used to formulate the cream are mentioned in Table 1.

A total of six formulations were prepared, three using methanolic extract and the remaining three using essential oil as an active ingredient. The quantity of stearic acid, white soft paraffin, and ethanol was changed in the formulations to evaluate the difference in formulation. M1-

M3 indicates formulations with methanol extract, and O1-O3 indicates volatile oil formulations throughout this study. The first set of cream formulations (M1, O1) has the same quantity of ingredients. Likewise, for the second (M2, O2)

Table 1: Components of Cream.

S.N.	Ingredients	Purpose of use
	Extracts of G. fragrantissima	Active ingredient
	Cetosteryl alcohol	Emulsion stabiliser, Surfactant
	Stearic acid	Emollient, Emulsifier, Lubricant
	White soft paraffin	Moisture barrier
	Ethanol	Skin penetration enhancer
	Potassium hydroxide	pH adjuster
	Sodium carbonate	pH adjuster
	Glycerin	Humectant
	Water	Solvent

and third (M3, O3) sets of cream formulations. The proportion of different ingredients used while making creams is mentioned in Table 2.

Evaluation of Cream

Different batches of formulated cream were evaluated on the following parameters.

Organoleptic properties

The appearance of the cream was analyzed by its color, pearlescence, and roughness[35].

Phase separation

Each sample was observed to determine the separation of oily and aqueous phases at given storage time and temperature[36].

Centrifugation test

Each sample was centrifuged immediately and observed to determine the separation of aqueous and oily phases at the given storage time and temperature. The centrifugation test was done after 0hr, 12hr, 24hr, 36hr, 48hr, 72hr, 7d, 14d, 21d and 28d of preparation. The centrifugation test was done for 10 minutes at 25 °C and 5000 rpm with a 2 g sample in centrifugal tubes[37].

Table 2: Quantity of different ingredients taken to make six different creams.

Ingredients	Methanol Extract (g)			Volatile Oil (g)		
	M1	M2	M3	O1	O2	O3
Active ingredient	0.5	0.5	0.5	0.5	0.5	0.5
Cetostearyl alcohol	15	15	15	15	15	15
Stearic acid	5.5	9	9	5.5	9	9
White soft paraffin	20	20	16.5	20	20	16.5
Ethanol	15	11.5	15	15	11.5	15
Sodium carbonate	2	2	2	2	2	2
Potassium hydroxide	2	2	2	2	2	2
Glycerine	40	40	40	40	40	40
Water	q.s.	q.s.	q.s.	q.s.	q.s.	q.s.
Total (g)	100	100	100	100	100	100

pH measurement

The pH was measured using a pH meter. The electrode was inserted into the sample 10 minutes before taking the reading at room temperature. Accurately 5 g of the sample was dispersed in 45 mL of water. The pH of the suspension was determined at 27 °C using a digital pH meter[38].

Determination of homogeneity

The formulations were tested by visual appearance and by touch for homogeneity[39].

Determination of emollience

Emollience, slipperiness, and the amount of residue left were checked after the application of a fixed amount of cream[36].

Stability testing

In this study, the formulation was divided into four samples separately, and these samples were kept at different storage conditions: at 8 °C (in refrigerator), at 25 °C (at room temperature), at 30 °C + 75 % RH (in real-time stability chamber), and at 40 °C + 75 % RH (in an accelerated stability chamber). These samples under different storage conditions were observed for a period of 28 days at definite time intervals. The samples were observed concerning changes in physical characteristics, liquefaction, and phase separation. Testing times were 0hr, 12hr, 24hr, 36hr, 48hr, 72hr, 7d, 14d, 21d and 28d[40].

Results and Discussion

Percentage yield and physical properties of the plant extracts

Table 3 demonstrates the physical properties and percentage yield of the methanolic extract and the essential oil. The percentage yield of essential oil in this study was found to be higher in comparison to the previous study, where the calculated value is 0.5 %[41]. The greater percentage yield of essential oil may be due to the extraction carried out during the harvesting season and the more favourable environment in which the plant grew. Other factors, such as method of extraction, external factors such as humidity, temperature, etc., can affect the yield percentage[42,43].

Physicochemical analysis of volatile oils

Organoleptic characteristics

The volatile oil was examined for its physical properties.

The oil was found to be colorless to nearly colorless with a strong characteristic odor and a pungent taste, which defines a strong feature of volatile oil derived from medicinal plants. The observations are found to be similar to the descriptions provided in the official pharmacopoeia and previous reports on essential oils of aromatic plants.

Table 3: Extraction parameters and properties.

Parameters	Extracting solvent	
	Methanol	Water
Extraction method	Cold Maceration	Hydrodistillation
Nature of extract	Semi-solid	Liquid
Color of extract	Yellowish-brown	Pale white
Percent yield	11.29 %	1.71 %

Phenolic compound identification

Phenolic compounds are the commonly reported constituents of volatile oils and serve as a preliminary confirmation of oil identity. The volatile oil gave a violet color in reaction with ferric chloride, confirming the presence of phenolic derivatives, a characteristic of wintergreen oil. G. fragrantissima oil is rich in methyl salicylate (>90 %), which is a phenolic ester[44].

Physicochemical analysis of leaf powder

The obtained values of various physiochemical parameters of the ground leaf are tabulated in Table 4. The alcohol-soluble extractive value was found to be 30.34 %, and the water–soluble extractive value was found to be 9.63 %, which confirmed that the selected plant extract is rich in alcohol-soluble phytochemicals such as flavonoid and phenolic compounds[45]. Similarly, total ash value and acid-

insoluble ash value were found to be 4.93 % and 1.37 %, respectively. These values suggest that the plant extract possesses a lower amount of inorganic matter and sand particles as contamination. The increase in total ash value indicates adulteration, contamination, and substitution[46]. The amount of water present in plant extract was found to be low (2.33 %), which indicates the stability of plant extract and reduced risk of growth of microorganisms during storage[45,47].

Table 4: Physiochemical parameters.

S.N.	Particular	Result (%)
1.	Alcohol-soluble extractive value	30.34
2.	Water- soluble extractive value	9.63
3.	Total ash determination	4.93
4.	Acid-insoluble ash determination	1.37
5.	Moisture content	2.33

Evaluation of vanishing cream

The organoleptic and physicochemical properties of the prepared vanishing creams have been summarized in Table 5. Rose pink color was observed in all methanol formulations, while the volatile oil formulations showed a pale white color. The pleasant odor of formulations signified good compatibility of the active extract with the formulation base. All formulations were found to be homogeneous, smooth, and non-greasy, reflecting good spread ability and emollient properties. The pH value ranged from 5.60 to 6.36, which is comparable to those reported for stable herbal and cosmetic formulations, which, when properly formulated, are unlikely to cause any skin irritation. Overall, both methanol extract and volatile oil-based formulations are suitable for topical cosmetic applications, meeting both the sensory and physicochemical requirements[48].

Stability testing, phase separation, and centrifugation test

No phase inversion was observed in the evaluation of different batches of cream over 28 days under either condition, further solidifying the homogeneity and stability of the emulsions, which have been reported in stable

Table 5: Evaluation of Vanishing Cream.

Parameters	Methanolic Extract			Volatile Oil		
	M1	M2	M3	O1	O2	O3
Appearance	Rose pink colour with pleasant odour			Pale white colour with pleasant odour		
Homogeneity	All 6 formulations are homogeneous, smooth, and consistent in both nature and application.					
Emollience	All 6 formulations left no residue on the skin.					
pH	6.36	6.27	6.09	6.29	5.87	5.60

cosmetic and herbal formulations [49,50]. Overall, the stability of both methanol and volatile oil formulations exhibited excellent resistance against phase inversion both during centrifugation and storage, indicating acceptable physical stability over the tested period of time.

Quantitative Analysis

Total phenolic content

The total phenolic content was expressed as Gallic Acid Equivalents (GAE). The phenolic content in the methanol extract, and the volatile oils were found to be 111.94 ± 1.36 mg GAE/g and 13.06 ± 1.36 mg GAE/g, respectively. An earlier study on G. fragrantissima reported a phenolic content of 77.06 ± 0.12 mg GAE/g for the methanol extract, which is lower than that obtained in our study [16]. Differential TPC values may be due to geographical variations that directly or indirectly affect the chemical composition of plants. Additional factors such as plant maturity, particle size, solvent polarity, and extraction procedures also play a major role and cause differences in observed results. Also, because of the predominant polar

nature of phenolic compounds, the extraction efficacy varies according to differential solvent polarity, making methanol the most effective solvent for TPC assessment.

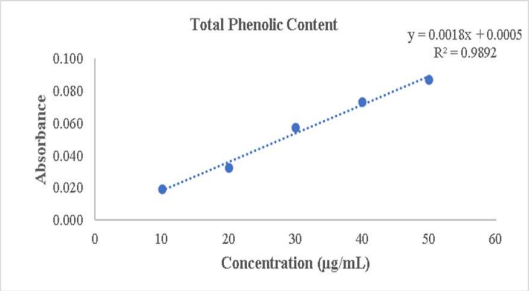

Figure 2: Calibration curve of gallic Acid.

Total flavonoid content

The total flavonoid content was expressed in terms of Quercetin Equivalents (QE). Among the tested samples, the methanol extract demonstrated the highest flavonoid content (50.02 ± 0.89 mgQE/g), whereas the essential oil demonstrated substantially lower flavonoid content of (9.55 ± 2.36 mg QE/g). As per an earlier study, the methanol extract of leaves of G. fragrantissima demonstrated flavonoid content value as 70.79 ± 0.01 mg QE/g[16]. This value, in comparison with the one obtained through our study, is higher, which may be due to various reasons. Dryness, heat, light, processing techniques, and other external factors are some of the most pronounced factors causing differential flavonoid biosynthesis as well as estimation. The polar to moderately polar nature of flavonoids, which are basically polyphenol compounds, supports the highest TFC value for methanol extract due to its polar nature and less volatile nature in comparison to essential oil, with a trace number of non-volatile components.

DPPH radical scavenging assay

The IC_{50} values, as shown in Figure 4, of the methanol extract and essential oil were calculated from the nonlinear regression using GraphPad Prism 10. The methanol extract showed the DPPH free radical scavenging potential with an IC_{50} (50 % inhibitory concentration) value of 59.80 ± 0.588 µg/mL. Similarly, essential oil also demonstrated radical scavenging activity with an IC_{50} value of 87.52 ± 0.908 µg/mL. The antioxidant activity of the methanolic extract and essential oil is found to be lower in comparison to the standard reference antioxidant quercetin, whose IC_{50} value is 10.11 ± 0.387 µg/mL. Secondary metabolites such as

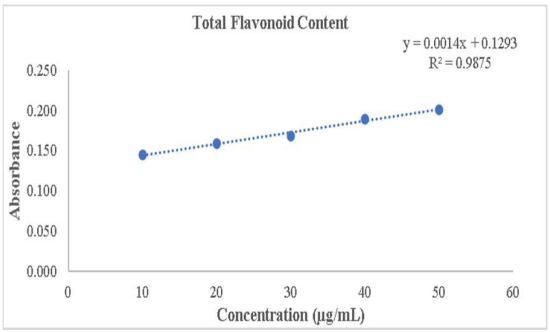

Figure 3: Calibration curve of Quercetin.

phenolics and flavonoids are widely gaining attention in natural products science due to their greater potential to neutralize free radicals [51,16].

A study conducted on the methanol extract of leaves of G. fragrantissima showed an IC_{50} value of 65.02 ± 0.12 µg/ml, and the essential oil demonstrated an IC_{50} value of 78.09 ± 0.13 µg/ml, which is almost comparable to the obtained experimental value of our study [16]. Overall, the G. fragrantissima demonstrates promising antioxidant potential, strengthening its application for further bioactivity assessment and utility in various fields of natural product chemistry.

Cytotoxicity in lung and breast cancer cell lines

The in vitro cytotoxic potential of methanol extract and essential oil was assessed against human lung cancer (A549) and breast cancer (MCF-7) cell lines, and results were expressed in CC_{50} (50 % cytotoxic concentration) value using nonlinear regression analysis in Graph Pad Prism 10. The results are presented below in fig.5, which clearly indicates that the methanol extract exhibited the highest anticancer potential against both human cancer cell lines as compared to the essential oil. Specifically, the methanol extract showed CC_{50} values of 41.68 ± 0.39 µg/mL in breast (MCF-7) and 57.09 ± 1.26 µg/mL in lung (A549) cells, whereas the essential oil exhibited significantly higher CC_{50} values of 71.96 ± 0.30 µg/mL in breast (MCF-7) and 91.96 ± 0.30 µg/mL in lung (A549). Similarly, the reference drug doxorubicin showed a CC_{50}

value of 15.56 ± 0.08 µg/mL in A549 and 12.47 ± 0.39 µg/mL in MCF-7. The greater cytotoxic potential of the methanol extract compared to the essential oil may be due

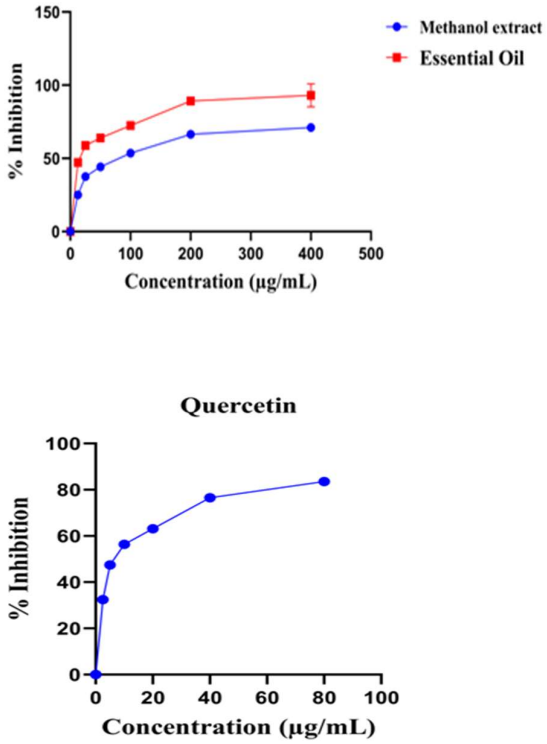

Figure 4: DPPH scavenging (%) vs. concentration of methanol extract, essential oil, and standard quercetin.

to the polarity of methanol, which enables efficient extraction of phenolic and flavonoid compounds present in the plant. These secondary metabolites are well known for their strong antioxidant and anticancer properties, including cell apoptosis, inhibition of cancer cell proliferation, and reduction of oxidative stress[52].

Conclusions

G. fragrantissima Wall. a medicinal plant with a wide range of biological activities, including antioxidant, anticancer, analgesic, and anti-inflammatory properties. The methanolic extract and essential oils of the plant showed different ranges of antioxidant and anticancer properties, which suggested its potential implication in the field of medicine. The preparation of herbal vanishing cream can be regarded as one step forward in realizing the traditional use of this plant with a scientific background and rationale. The cream formulation relied mostly on methyl salicylate, being the main active component of G. fragrantissima extract. Methyl salicylate is proclaimed to be very useful against pain, inflammation, etc. Hence, our formulation was aimed at treating minor aches, pain, and inflammation caused by strain, sprain, arthritis, etc.

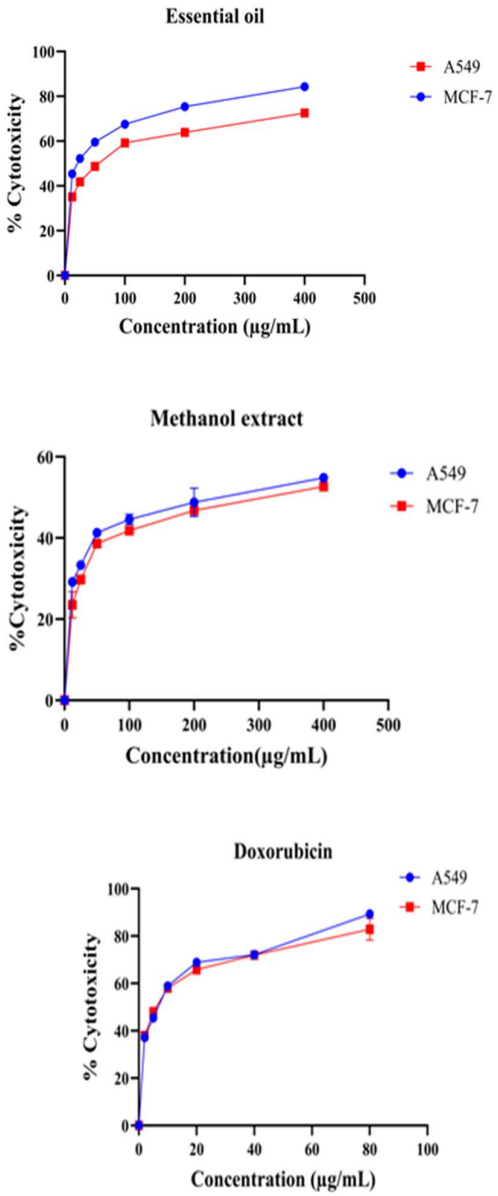

Figure 5: % Cytotoxicity Vs. Concentration graph of the essential oil, methanol extract, & standard doxorubicin.

Further studies are needed to investigate the safety and efficacy of this herbal cream in humans.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

We would like to sincerely thank the Central Department of Chemistry, Tribhuvan University, Department of Chemistry, Tri-Chandra Multiple Campus, and Meera Biotech Pvt. Ltd. for the necessary laboratory support and tech Pvt. Ltd. for the necessary laboratory support and facilities provided for the completion of this project.

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