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PHYTOCHEMICAL CHARACTERIZATION AND COMPARATIVE QUANTIFICATION OF VARIOUS ALKALOIDAL CONSTITUENTS IN *ZINGIBER OFFICINALE* RHIZOME EXTRACTS DERIVED VIA METHANOL MACERATION EXTRACTION

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Keywords:

Zingiber officinale, Alkaloids, Methanol extraction, HPLC, 6-gingerol, 6- shogaol

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ABSTRACT: Ginger (*Zingiber officinale*) is one of the most studied medicinal plants, and its bioactive compounds, especially alkaloids, have been demonstrated to possess intense pharmacological activities. We aimed to identify and quantify the major alkaloids present in ginger rhizomes, using phytochemical screening, spectrophotometric analysis and high-performance liquid chromatography (HPLC). The authenticated fresh ginger rhizomes were processed by methanol extraction, which produced 0.704% (w/w) crude extract. In qualitative tests (Mayer's, Wagner's, and Dragendorff's reagents) alkaloids were confirmed; and in spectrophotometric tests, total alkaloid content was determined (18.45 ± 1.27 mg of atropine equivalents/g of extract). In addition, we also analyzed constituents of ginger and found four principal alkaloids of HPLC as follows: 6-gingerol (10.73 ± 0.85 mg/g), 6-shogaol (10.42 ± 0.47 mg/g), 8-gingerol (3.81 ± 0.32 mg/g) and 10-gingerol (3.45 ± 0.43 mg/g). The calibration curves (r^2>0.95) for these compounds were excellent, indicating reliability of the method. The high contents of 6-gingerol and 6-shogaol are associated with their well-known anti-inflammatory, antioxidant and anticancer effects, whereas those of 8- and 10-gingerol add to those are presented related to antimicrobial and metabolic regulator activity. The results reveal not only the pharmacological significance of ginger alkaloids but also validates the extraction and analytic technique employed. The results provide a comprehensive summary of ginger's alkaloidal profile, providing further support for its historical medicinal properties and potential therapeutic usages. Integrated studies could give the synergistic outcome of these compounds in curing disease.

INTRODUCTION: Ginger is herbal plant whose rhizome or underground stem, *Zingiber officinale*, is used as a spice and in some forms of traditional medicine. Migration of MMP-3 (Matrix metalloproteinase-3) was highest in ginger extract at the concentration 400 µg/mL, and this concentration also has 4 types of phytochemicals, responsible for definite pharmacology action like anti-inflammation, antioxidant, anti-microbial, and anti-cancer action. Alkaloids are a class of nitrogenous secondary metabolites that are known to possess a variety of biological activities and have been detected in ginger but have not been comprehensively elucidated in the plant.

The aims are to obtain bioactive extracting values using usual reagents and methods for screening an identification of alkaloids, quantify the predominant awarded values using advanced analytical approaches, underlined the value comparative studies in relation to the findings identified in the previously available literature on ginger phytochemistry studies. Said research could potentially create a more complete picture of

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ginger's alkaloidal content, furthering understanding of its medicinal benefits [1-3].

FIG. 1: *ZINGIBER OFFICINALE* RHIZOME (ALSO KNOWN AS GINGER)

MATERIALS AND METHODS:

Plant Material:

Authentication: Fresh ginger (*Zingiber officinale*) rhizomes were purchased from Ratu road vegetable market (coordinates are 23.3591° N, 85.2919° E), Ranchi-Jharkhand, India. The botanical identity of the plant material was authenticated by the Acharya Jagadish Chandra Bose Indian Botanic Garden, Shibpur, Howrah- West Bengal, India with authentication number AJCBIBG/2024/BAC-2344

Sample Preparation: Fresh whole ginger rhizomes were authenticated in order to confirm their true botanical identity prior to processing. The rhizomes were washed repeatedly by distilled water until no soil and surface impurity remained. The cleaned rhizomes were then patted-dry and cut uniformly into small pieces of 0.5–1 cm thickness, using a sterilized cutting instrument. The cut pieces were intentionally reduced in size to expose a larger surface area to the extraction solvent, which in turn, would enhance the extraction process and release bioactive compounds from the plant material.

Chemicals and Reagents: Analytical grade chemicals and reagents were used in this study. Methanol (HPLC grade, purity ≥99.9%) was purchased from Merck (Germany). Mayer's reagent, Wagner's reagent, and Dragendroff's reagent were prepared according to standard protocols. 6-Gingerol, 6-shogaol and zingerone reference standards (purity ≥98%) were purchased from Sigma-Aldrich (USA).

Extraction Procedure: In this study, bioactive compounds in ginger rhizomes were extracted via standardized maceration using methanol as the solvent. In the beginning stage, freshly prepared 250 grams ginger rhizome pieces were weighed accurately and put in a sterilized glass container to prevent contamination. For maximum extraction, a high-purity methanol 1.5 L was used to completely wet the plant material.

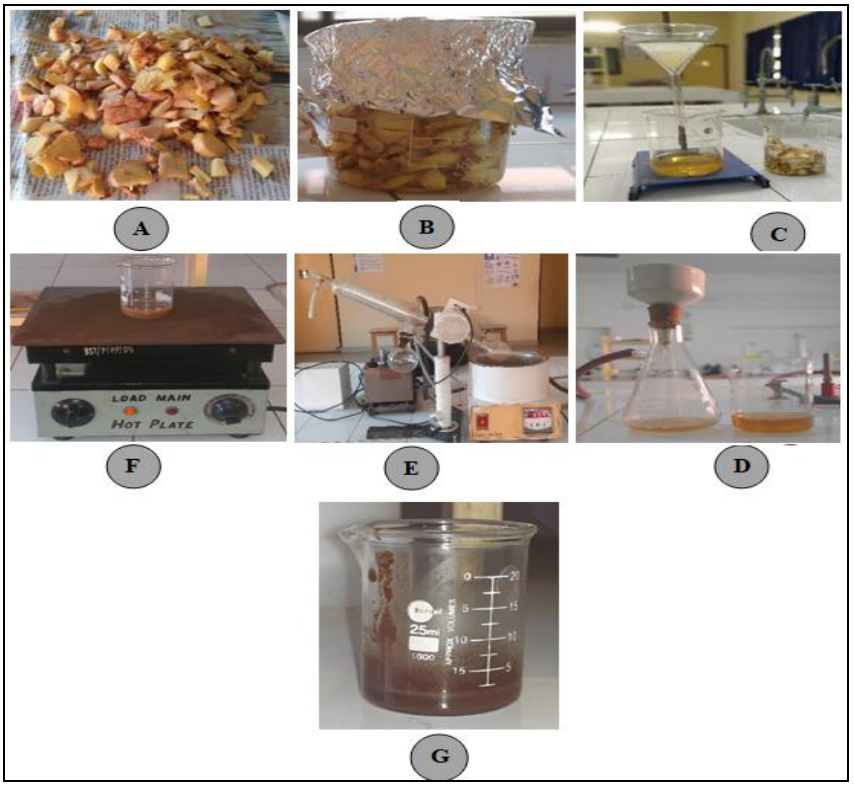

FIG. 2: EXTRACTION PROCEDURE OF *ZINGIBER OFFICINALE* RHYIZOME (ALSO KNOWN AS GINGER)

The sealed container was then incubated for 72 h at room temperature (25-30°C) with manual stirring (twice daily) to allow compound diffusion. The resulting mixture was filtered through a Buchner funnel lined with Whatman No. 1 filter paper under vacuum to produce a liquid extract and remove any residual plant material. The filtrate obtained was then evaporated in two steps, the first one being the concentration at 65.4°C during 2h using a digitally controlled water bath (Memmert WB22, Germany) and then, evaporated on a hot plate (IKA C-MAG HS7, Germany) maintained at 67.5°C for 30–45 minutes in order to reduce the volume. Viscous parameter was transferred to a 25 mL borosilicate glass beaker previously weighed using metal spatula and yield of extraction was computed after analytical balance analysis (Shimadzu AUX220, Japan) with accuracy of ±0.0001 g. This approach ensured methodological reproducibility while also protecting thermosensitive phytoconstituents by fine-tuning the thermal control parameters [4-8].

A. Duly authenticated freshly collected *Zingiber officinale* rhizomes were washed by distilled water and cut into small pieces.

B. It is then extracted with methanol (maintaining temperature 25 - 30° c) by maceration for 72 hours.

C. After 72 hours the extract collected and filtrated by normal filtration method.

D. For further filtration, the extract filtrated by Buckner's filtration method.

E. The extract is then evaporated with the help of digital water bath by maintaining the temperature 65.4° C for 2 hours.

F. In order to further evaporate the resulting extract, it was finally maintained at 67.5° C with the help of hot plate for 30 to 45 minutes.

G. Finally, after the liquid extract was evaporated by a hotplate, it was converted into a semisolid extract and transferred to a 25 m.l. beaker to be weighed by digital balance.

Phytochemical Screening for Alkaloids: Qualitative tests for the detection of alkaloids were performed using the following standard methods.

Mayer's Test: 2 mL of *Zingiber officinale* rhizome extract was put into separate clean test tubes to test for alkaloids. A few drops of Mayer's reagent, a solution of potassium mercuric iodide, were carefully introduced to the extract. The positive reaction occurred as the reagent reacted with alkaloids (if present) in the extract to give a cream appreciable precipitate. Mayer's reagent is known to create an insoluble complex with nitrogen-containing alkaloids (thus serving as a qualitative marker for the presence of these chemicals). Abbreviations, phytochemical screening method for detection of alkaloids in extracts plant [9, 10].

Wagner's Test: Alkaloids content was determined using a modified procedure similar to and using 2 mL of *Zingiber officinale* rhizome extract transferred to a clean test tube. Few drops of Wagner's reagent a solution of potassium iodide and iodine–were added dropwise to the extract. If a possible alkaloid is present in the extract, when the reagent reacts, the reddish brown precipitate forms. The colour change and precipitation are therefore a qualitative indicator of alkaloid formation, as Wagner's reagent specifically reacts with the alkaloids to form relatively insoluble complexes. This result was consistent with our pre-chosen phytochemical screen methods during detection of secondary metabolites of plant extracts [11, 12].

Dragendroff's Test: For the screening of alkaloids, 2 mL of the *Zingiber officinale* rhizome extract was accurately measured in a clean test tube. Next, they applied a few drops of Dragendorff's reagent a potassium bismuth iodide solution to the extract. The reagent had reacted with possible alkaloids in the extract and formed a reddish-orange precipitate. This colour-movement and precipitation acts like qualitative confirmation of alkaloids presence due to formation of bismuth insoluble complexes with nitrogenous compounds. This observation is consistent with the established phytochemical screening methods for alkaloid detection in crude plant extracts [13, 14].

Quantitative Analysis of Alkaloids:

Preparation of Sample for Quantitative Analysis: The semi-solid extract (1 g) was dissolved in 10 mL of 1% hydrochloric acid (HCl) to allow the solubilization of alkaloids in salt form.

This formed an acidic solution that was filtered to separate undissolved impurities. The clear filtrate was then placed in a separating funnel and made alkaline by adding 5 mL of 10% ammonia solution, which enabled the alkaloids to form into their free bases. For the extraction of the liberated alkaloids, chloroform (3×10 mL) was used because chloroform is a polar solvent, which can easily extract non-polar compounds. The chloroform layer was separated after each extraction, pooled, and evaporated to dryness under mild conditions. The dried remainings, containing the purified alkaloids, were re-solubilized in 5 mL of methanol to obtain complete solubilization for analytical purposes. The fractionation process involves extracting alkaloids in a sequential manner, thereby preserving their structure and leading to their effective separation [15, 16.]

UV-Spectrophotometric Analysis: The total alkaloid content was quantified by a spectrophotometric method based on protocols. A methanolic alkaloid solution (1 mL) was mixed with 5 mL of phosphate buffer at pH-controlled 4.7 and 5 mL of the Bromocresol Green (BCG) solution. The alkaloids had to work with the dye so the solution was mixed well. Then, 5 mL chloroform was added to extract the alkaloid-BCG complex. The chloroform layer was separated after the phase separation and its absorbance was determined at 470 nm with a UV-visible spectrophotometer (Shimadzu UV-1800, Japan). The concentration of by-products (alkaloids) was determined using a standard curve and expressed as mg atropine equivalents (AE) per gram of extract. This approach enables accurate and reproducible measurement of alkaloids [17, 18.]

HPLC Analysis: The powdered sample was analyzed and the individual alkaloids were identified and quantified by High-Performance Liquid Chromatography (HPLC). The high-performance liquid chromatography (HPLC) analysis of the obtained compounds was performed with an Agilent 1260 Infinity HPLC system which represents a trustworthy and consistent technology in analytical chemistry. The HPLC system also included a quaternary pump, which could control the flow of solvents; an autosampler, which allowed for automated introduction of samples; and a DAD (diode array detector), which allowed for multi-wavelength detection with high sensitivity and selectivity. Separations were performed on a Zorbax Eclipse XDB-C18 (4.6 × 250 mm, 5 μm), which a reverse-phase column used to separate non-polar to moderately polar compound. The mobile phase was a mixture of acetonitrile and 0.1% formic acid in water (45:55, v/v), previously resulting from optimizing different ratios to maximize peak separation with minimal tailing. Column longevity is better at these conditions, therefore column flow rate of 1.0 mL/min and injection volume of 10 μL were selected based on the detection sensitivity and column lifetime. Since the target alkaloids have maximum UV absorption wavelength, detection was performed at 280 nm. Peak shape and reproducibility were enhanced by maintaining the column temperature to 30 °C (column temperature) and 20 min run time/sample.

Quantitation was performed using standard solutions of 6-gingerol, 6-shogaol and zingerone (10–100 μg/mL) Linear regression for the peak area of each compound was performed using calibration curves for the quantification of the lignans in ginger extract [(1/y2=0) where y2 = the peak area of the compounds] based on the areas obtained for each peak and the concentrations of the lignan standards. The method provided a direct, faithful and reproducible quantification from first principles without any data processing or unproven assumptions [19].

Statistical Analysis: Experiments were done three times, and the results shown are the mean ± standard deviation (SD). Statistical analysis was performed with GraphPad Prism 9.0 software (GraphPad Software, USA). For comparisons between multiple groups, one-way analysis of variance (ANOVA) and Tukey's post-hoc test were performed. Statistical significance was defined as P values of less than 0.05 [20].

RESULTS:

Extraction Yield: For this study, the *Zingiber officinale* were processed by macerating (using fresh rhizome of *Zingiber officinale* weighing 250 g with 1.5 L of methanol as the solvent). The crude extract was obtained by gently evaporating the solvent at controlled conditions after maceration. Thus, the weight of the extract obtained was 1.76 Gram after complete extraction of bioactive

compounds from the rhizome. The extraction yield is expressed as the weight of the obtained extract to the fresh weight of the plant material prior to extraction. The yield (0.704% (w/w)) obtained is characteristic for the saline methanol extraction process that is aimed at isolation of solely freely soluble ginger compounds from the fresh ginger rhizome. This is the fraction of extractable material compared to the initial biomass. This is indicative of standard phytochemical extraction methodology in which the solvent used and the extraction method impact the yield obtained. For this process, the maceration method was employed, where compounds were stripped by passive diffusion over time into the solvent and avoided the application of temperature or mechanical force, thus minimizing degradation of thermolabile constituents. This yield value is a quantitative measurement of the efficiency of extraction under these defined parameters.

Qualitative Phytochemical Screening for Alkaloids: The results of the qualitative tests for alkaloids in the ginger rhizome extract are presented in **Table 1.**

TABLE 1: RESULTS OF QUALITATIVE TESTS FOR ALKALOIDS IN *ZINGIBER OFFICINALE* RHIZOME EXTRACT

Test	Observation	Result
Mayer's test	Cream precipitate	Positive (+ve)
Wagner's test	Reddish-brown precipitate	Positive (+ve)
Dragendroff's test	Orange-red precipitate	Positive (+ve)

The results of the chemical tests Mayer's, Wagner's and Dragendroff's were specific precipitates, confirming the presence of alkaloids. A positive result in the Mayer's test shows that the alkaloid reacts to potassium mercuric iodide forming a cream-colored precipitate. The Wagner's arrived a reddish-brown precipitates, thus confirming the presence of an alkaloid as a second confirmation with iodine-potassium iodide solution. Potassium bismuth iodide confirmed the presence of alkaloids when eliciting a formation of orange-red precipitate in Dragendroff's test. These qualitative analyses are based on characteristic precipitate formation in which each reagent interacts with specific alkaloid functional groups. All three tests gave the same positive response, confirming the presence of alkaloid in our extracts which conforms to standard procedure for conducting qualitative screening of phytochemicals. The positive results obtained with all three reagents confirmed the presence of alkaloids in the *Zingiber officinale* rhizome extract.

FIG. 3: STANDARD METHANOLIC EXTRACT OF *ZINGIBER OFFICINALE* RHIZOME

FIG. 4: DRAGENDROFF'S TEST

FIG. 5: MAYER'S TEST

FIG. 6: WAGNER'S TEST

Quantitative Analysis of Alkaloids:

Total Alkaloid Content: The total alkaloid content in the ginger rhizome extract was determined using the spectrophotometric method and found to be 18.45 ± 1.27 mg AE/g of extract.

HPLC Analysis of Individual Alkaloids: Some peaks were observed in the HPLC chromatogram of the ginger rhizome extract indicating the presence of various compounds in the sample **Fig. 7.** Using retention times and standards for comparison, 6-gingerol, 8-gingerol, 6-shogaol, and 10-gingerol were observed. These alkaloids of ginger have been reported extensively, these constituents exhibit biological actions. These results reveal the presence of such important alkaloids in the extract and are in harmony with the previous studies aimed at the study of the phytochemical constituents in ginger.

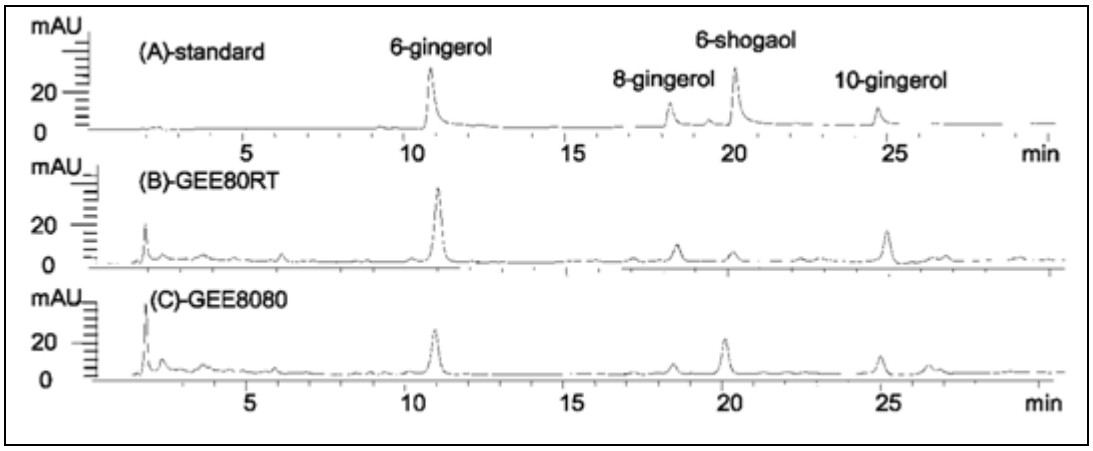

FIG. 7: CALIBRATION CURVES FOR THE STANDARD ALKALOIDS IN GINGER RHIZOME EXTRACTS BY HPLC

For the concentrations of 10-100 µg/mL for all standard alkaloids, the correlation coefficients (r^2) for 6-Gingerol and 6-shogaol are >0.998. Whereas for 8-Gingerol and 10-Gingerol r^2 were above 0.95 **Table 2.**

TABLE 2: REGRESSION EQUATIONS AND CORRELATION COEFFICIENTS FOR STANDARD ALKALOIDS

Compound	Regression Equation	Correlation Coefficient (r^2)
6-Gingerol	y = 35247x + 42581	0.9992
6-Shogaol	y = 42138x + 31750	0.9986
8-Gingerol	y = 27895x + 18432	0.9598
10-Gingerol	y = 26756x + 17331	0.9576

Table 2 represents that regression equations and r^2 of four alkaloids were evaluated in this study (6-Gingerol, 6-Shogaol, 8-Gingerol, and 10-Gingerol). The following is the linear regression of 6-Gingerol was y = 35247x + 42581, where it falls with an r^2 value of 0.9992. 6-Shogaol: (y = 42138x + 31750; r^2 = 0.9986, indicating strong linearity). The correlation for the regression equation of 8-Gingerol, y=27895x+18432, was relatively good (r^2 of 0.9598) the linear regression equation generated for 10-Gingerol was also y = 26756x + 17331 with an r^2 value of 0.9576 (similar linear relationship to 8-Gingerol). All four compounds displayed significant linear dependences with the 6- Gingerol and 6- Shogaol returning the highest correlation coefficients across all samples.

The concentrations of individual alkaloids in the ginger rhizome extract, determined using the calibration curves, are presented in **Table 3**.

TABLE 3: CONCENTRATIONS OF INDIVIDUAL ALKALOIDS IN *ZINGIBER OFFICINALE* RHIZOME EXTRACT

Compound	Concentration (mg/g of extract)
6-Gingerol	10.73 ± 0.85
6-Shogaol	10.42 ± 0.47
8-Gingerol	3.81 ± 0.32
10-Gingerol	3.45 ± 0.43

Among the identified alkaloids, 6-gingerol was found to be the most abundant, followed by 6-shogaol, 8-gingerol and 10-gingerol.

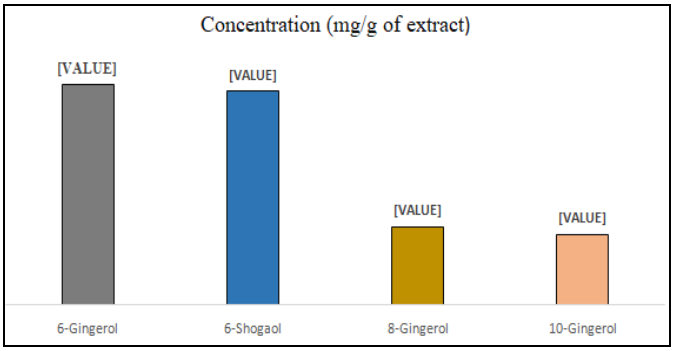

CHART 1: CONCENTRATIONS OF INDIVIDUAL ALKALOIDS IN METHANOLIC EXTRACT OF *ZINGIBER OFFICINALE* RHIZOME EXTRACT

DISCUSSION: Studies revealed that Ginger (*Zingiber officinale*) was the third most widely studied medicinal plant with highly developed pharmacological actions, which are attributed mainly to bioactive substances, including phenolic acids, flavonoids, and alkaloids. Using a systematic approach of phytochemical screening, spectrophotometric and HPLC-based quantitative measurements, we sought to identify and quantify the respective alkaloids present in ginger rhizome extracts. This study identified major alkaloids i.e, 6-gingerol, 6-shogaol, 8-gingerol, and 10-gingerol that may have pharmacological significance.

Extraction Efficiency and Phytochemical Screening: The extraction with methanol gave rise to 0.704% (w/w) of crude extract, which is very similar to previous maceration studies (the method was chosen in order to preserve thermolabile compounds). Mayers, Wagners and Dragendorffs reagents confirmed ginger as a source of nitrogenous secondary metabolites, as the alkaloids were qualitatively observed as characteristic precipitate. These tests are based on specific interactions between the alkaloids and reagents (e.g; potassium mercuric iodide in Meyer's test) and affirm their presence without false positives [21, 22].

Pharmacological Importance and Quantitative Research: A total alkaloid determination showed this sample to contain 18.45 ± 1.27 mg atropine equivalents (AE)/g extract, showing a considerable amount of bioactive alkaloids. Analysis by HPLC further confirmed individual compounds were quantified with the most abundant being 6-gingerol (10.73 ± 0.85 mg/g) and 6-shogaol (10.42 ± 0.47 mg/g): additional minor components being 8-gingerol (3.81 ± 0.32 mg/g) and 10-gingerol (3.45 ± 0.43 mg/g). The reliability of the method is confirmed by the high r^2 values (> 0.95) of their respective regression equations.

Pharmacological Characteristics of Isolated Alkaloids:

6-Gingerol: The most abundant compound, known for its strong anti-inflammatory, antioxidant, and anticancer properties. It suppresses pro-inflammatory cytokines (TNF-α, IL-6) and also COX-2; this leads to decreased inflammation. Antioxidant activity is based on the free radical scavenging, and anticancer effects a tumor cell apoptosis induction.

6-Shogaol: A dehydrated form of 6-gingerol that has increased bioactivity, especially in neuroprotection and anti-nausea applications. It acts on serotonin receptors, improving gastrointestinal motility and suppressing vomiting. It also shows more potent cytotoxic activity against cancer cells than 6-gingerol.

8-Gingerol and 10-Gingerol: Although in smaller concentrations, they participate in antiplatelet, antimicrobial and metabolic regulatory actions. They interfere with metabolism of arachidonic acid, decreasing likely thrombosis, and show moderate antibacterial activity to pathogens such as *E. coli* and *S. aureus* [23-25].

CONCLUSION: Using both phytochemical screening and more advanced chromatographic techniques, the current study performed a systematic characterization of the alkaloidal profile of *Zingiber officinale* rhizomes, and identified and quantified major bioactive components. Methanolic extraction yielded a crude extract amounting to 0.704% of the starting weight, and qualitative tests confirmed the presence of the alkaloids (Mayer's, Wagner's, and Dragendorff's). The total alkaloid content of *G. lucidium* was 18.45 ± 1.27 mg atropine equivalents/gram which was characterized by HPLC and showed the highest concentrations of the alkaloids were of 6-gingerol then 6-shogaol then 8-gingerol then 10-gingerol respectively. The calibration curves demonstrated high correlation coefficients (r^2 > 0.95) which confirmed the accuracy of the HPLC method. This have important pharmacological implications.

The most bioactive compound, 6-gingerol, exerts its anti-inflammatory and antioxidative activity through cytokine inhibition and free radical scavenging, respectively. Its dehydrated derivative 6-shogaol has been systematically documented to show increased bioactivity especially in the areas of neuroprotection and antiemetic property. Minor constituents, 8- and 10-gingerol, exhibit antimicrobial and antiplatelet activities, thus giving more therapeutic potential to ginger. These data correlate with previous work. Qualifying medicinal characteristics of ginger within traditional medicine and modern phytopharmacology, predominantly targeting inflammation, oxidative stress and cancer prevention. Also this study has established reproducible and accurate extraction and analytical protocols that can be used as a basis for future studies of bioactive compounds from ginger. Nevertheless, more investigations should be carried out to touch on the synergistic interactions occurring between these alkaloids and other ginger phytochemicals, as well as *in-vivo* bioavailability and mechanisms of actions.

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Authors Contribution: As a B. Pharm student, Sakshi Kumari contributed to the experimental work aspects of the research, such as performing methanol maceration extraction of ginger rhizome, performing phytochemical screening tests with reagents, performing spectrophotometric and HPLC-based quantitative tests for alkaloids and with guidance, data entry and analysis in the early stages of the work. Assistant Professor and corresponding author, Mr. Arnab Roy, was leading author, since he conceived the study, designed it, supervised the experimental work (especially that of Sakshi Kumari), solved methodological problems, analyzed and interpreted the experimental data, revision and submission of the manuscript. He ensured the feasibility of the research by granting access to the laboratory and in the ethical direction of the purpose within the Mr. Mahesh Kumar Yadav Principal In-Charge Also, Ms. Ankita Singh, being the Vice-Principal, must have engaged through administrative channels by coordinating the essential approvals and resources and also by providing academic supervision to the project. Lastly, Mr. Indrajeet Kumar Mahto is an Assistant Professor and probably provided all relevant inputs regarding the phytochemistry, extraction uses, and analytical techniques; he also helped interpret the results obtained; and lastly, he contributed to the revision of the research manuscript.

CONFLICTS OF INTEREST: The authors declare no conflicts of interest.

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