Journal of Advanced Zoology

ISSN: 0253-7214
*Volume **44** Issue **S-5 Year 2023** Page **2403:2411***

Polyphenols, Flavonoids, Mineral Elements, and Biological Activities of Ginger and Cinnamon Essential Oil and Extracts as Regulated by Their Isolation Procedures

Mariam Aysha A[1], K Aishwarya[1], V S Subashini[1], M. Sangeetha[1], Farha Naaz[2], Jytosna Upadhyay[2], Roshan Kumar[2], Prachi Sood[2]

[1]Assistant Professor, Department of Chemistry, Thassim Beevi Abdul Kader College for Women,Kilakarai, Ramanathapuram.
[2]Assistant Professor, Department of Pharmaceutics, Shree Dev Bhoomi Institute of Education Science and Technology (SDBIT), Dehradun, INDIA.

**Corresponding author's E-mail: prachisood125@gmail.com *Submitting author's Email: rjroshan244@gmail.com*

Article History

Received: 06 June 2023
Revised: 05 Sept 2023
Accepted: 15 Nov 2023

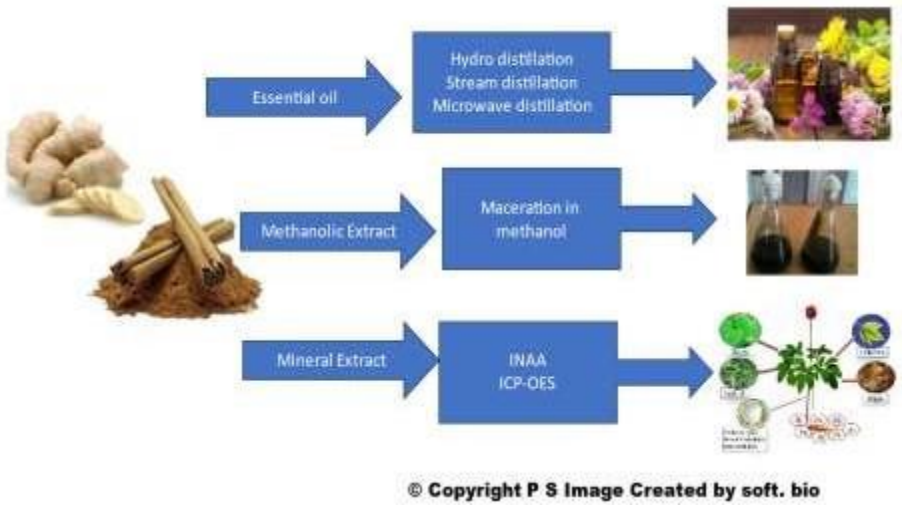
Abstract

Our research compared the chemical make-up of wild Ginger and cinnamon, including their essential oils (EOs), total phenol, and total flavonoid, for their antioxidant and antibacterial effects in vitro. The mineral (nutritional and poisonous) components of the plant were also identified in this investigation. Hydro distillation (HD), steam distillation (SD), and microwave-assisted distillation (MAD) were used to extract the EOs, and gas chromatography with flame ionization detection (GC-FID) and mass spectrometry detection (GC-MS) were used to examine them. 2,2-diphenyl-1-picrylhydrazyl (DPPH) was used to evaluate the EOs' antioxidant properties. The essential oil was analyzed, and twenty-six components were found to make up 97.73% of the oil with a yield of 0.202%. The primary components were pulegone (74.81%), menthone (13.01%) and piperitone (3.82%). Neutron activation analysis (INAA) and inductively coupled plasma optical emission spectrometry (ICP-OES) were used to detect twenty-one elements, including macro- and micro-elements (Ba, Br, Ca, Cl, Co, Cr, Cs, Eu, Fe, K, Mg, Mn, Mo, Na, Rb, Sb, Sc, Sr, Th, U, and Zn), with the mineral element concentration being very close to the FAO recommendation.

Keywords: *Metal ion, Polyherbal, DPPH, Flavonoids*

1. Introduction

Graphical Abstract



Researchers nowadays are paying close attention to plants used for food and medicine because of the wide variety of useful chemicals found in plants, including polyphenols, flavonoids, proteins, and vitamins (1, 2). These polyphenols are secondary metabolites that are increasingly being used in allopathic medicine due to their vital function in human health maintenance as antioxidants, anticancer, and antibacterial agents. The aromatic rings of these compounds, which include hydroxyl groups in various locations (1, 3), are responsible for their bioactivity. Polyphenols' therapeutic effects can be enhanced by combining them with macromolecules, but they are not without problems such as instability, low bioavailability, oxidation and destruction from light and heat, and poor membrane permeability (4). Polyphenols can be made more stable through direct polymerization of monomers or through coupling with macromolecules. Free radical and step-growth enzyme-catalyzed processes were among the direct polymerization methods they explored. Polymers with molecular weights between 890 and 77,000 (5) have been produced from polyphenols such catechin, quercetin, epicatechin, EGCG, rutin, tannic acid, and ferulic acid. Several potential medical applications have been demonstrated, including the suppression of XO, proteinase, and LDL oxidation; the delivery of medications; and the treatment of cancer, germs, and fungi. Polymerization preserves or boosts the antioxidant action of polyphenols. By esterification, amidation, free radical grafting, and enzyme-assisted grafting, polyphenol polymer conjugates have been produced (6). Gallic acid, ferulic acid, caffeic acid, tannic acid, EGCG, catechin, quercetin, curcumin, and epicatechin are all examples of polyphenols that have been conjugated with synthetic and natural polymers.

Polyphenols' antioxidant action is preserved or even increased through conjugation to polymers, just as it is through direct polymerization. Bone regeneration, skin care, Alzheimer's disease, diabetes, LDL oxidation, and enzyme inhibition; antioxidant, anticancer, antibacterial, and anti-inflammatory therapies are only some of the many positive therapeutic applications of polyphenol polymers (8). In this investigation, polyphenolic extracts from medicinal plants were used as antioxidants and antimicrobials due to the fact that many microbes have become resistant to conventional medicines or that conventional medicines are ineffective against oxidative stress or various cancers (9-13).

Polyphenol complexes' antioxidant activity helps to ward off conditions like respiratory disease, diabetes, neurological disease, cancer, and cardiovascular disease (3, 14, 15). The phenolic compounds found in plants have been shown to have potent antimicrobial, antifungal, and antiviral activity, leading to their widespread use in the production of pharmaceutical preparations (16–20) thanks to their safety, versatility, and efficacy even against drug-resistant microbes. It has also been found in recent studies (21, 22) that these medicinal herbs have anticancer activity against malignant cell lines and that they work synergistically with various cancer medicines. Flavonoids and phenolic acids are responsible for these beneficial effects; flavonoids make up about two-thirds of the dietary polyphenols in plant-based diets. But phenolic acids make up a third of the total. Because of their antioxidant qualities and potential health benefits (23), these phenolic acids are gaining increasing attention in the scientific community. Humans ingest roughly 25 mg of phenolic acids everyday (24), most of which comes from the fruits, coffee, spices, and vegetables we eat regularly.

The research on a plant origin for a therapeutic medicinal material discovery or development is expensive and is a demanding effort [19]. It takes at least ten years and anywhere from $100-$360 million to bring a new medicine to market. Only around one-fourth of the 10,000 chemicals studied up until 1992 were actually usable as medicines. The National Cancer Institute evaluated 50,000 plant extracts for biological activity and discovered three biologically active compounds for treating human immunodeficiency virus and three biologically active compounds for anticancer activity [17]. They necessitated fundamental understanding of chemistry, biology, chemistry, pharmacology, and toxicity. The importance of each of these fields should not be downplayed in favor of the others. Organic chemistry, ethnography, biotechnology, agronomy, and basic pharmacological expertise all play significant roles in the development of any novel plant-based pharmaceutical [20]. Herbal teas or the creation of pharmaceutical powder tablets, tinctures, capsules, fluid extract, standard enhance, or crude extract are all viable options for therapeutic treatment (at home) when a medicinal plant is discovered. Medicinal drugs like ergotamine (via its precursor diogenin), digoxin, and quinine are all derived from plants that have active natural compounds that may be extracted and purified by an extraction procedure [21].

2. Materials And Methods

Collection of plants

Researchers nowadays are paying close attention to plants used for food and medicine because of the wide variety of useful chemicals found in plants, including polyphenols, flavonoids, proteins, and vitamins (1, 2). These polyphenols are secondary metabolites that are increasingly being used in

allopathic medicine due to their vital function in human health maintenance as antioxidants, anticancer, and antibacterial agents. The aromatic rings of these compounds, which include hydroxyl groups in various locations (1, 3), are responsible for their bioactivity. Polyphenols' therapeutic effects can be enhanced by combining them with macromolecules, but they are not without problems such as instability, low bioavailability, oxidation and destruction from light and heat, and poor membrane permeability (4). Polyphenols can be made more stable through direct polymerization of monomers or through coupling with macromolecules. Free radical and step-growth enzyme-catalyzed processes were among the direct polymerization methods they explored. Polymers with molecular weights between 890 and 77,000 (5) have been produced from polyphenols such catechin, quercetin, epicatechin, EGCG, rutin, tannic acid, and ferulic acid. Several potential medical applications have been demonstrated, including the suppression of XO, proteinase, and LDL oxidation; the delivery of medications; and the treatment of cancer, germs, and fungi. Polymerization preserves or boosts the antioxidant action of polyphenols. By esterification, amidation, free radical grafting, and enzyme-assisted grafting, polyphenol polymer conjugates have been produced (6). Gallic acid, ferulic acid, caffeic acid, tannic acid, EGCG, catechin, quercetin, curcumin, and epicatechin are all examples of polyphenols that have been conjugated with synthetic and natural polymers.

Polyphenols' antioxidant action is preserved or even increased through conjugation to polymers, just as it is through direct polymerization. Bone regeneration, skin care, Alzheimer's disease, diabetes, LDL oxidation, and enzyme inhibition; antioxidant, anticancer, antibacterial, and anti-inflammatory therapies are only some of the many positive therapeutic applications of polyphenol polymers (8). In this investigation, polyphenolic extracts from medicinal plants were used as antioxidants and antimicrobials due to the fact that many microbes have become resistant to conventional medicines or that conventional medicines are ineffective against oxidative stress or various cancers (9-13).

Polyphenol complexes' antioxidant activity helps to ward off conditions like respiratory disease, diabetes, neurological disease, cancer, and cardiovascular disease (3, 14, 15). The phenolic compounds found in plants have been shown to have potent antimicrobial, antifungal, and antiviral activity, leading to their widespread use in the production of pharmaceutical preparations (16–20) thanks to their safety, versatility, and efficacy even against drug-resistant microbes. It has also been found in recent studies (21, 22) that these medicinal herbs have anticancer activity against malignant cell lines and that they work synergistically with various cancer medicines. Flavonoids and phenolic acids are responsible for these beneficial effects; flavonoids make up about two-thirds of the dietary polyphenols in plant-based diets. But phenolic acids make up a third of the total. Because of their antioxidant qualities and potential health benefits (23), these phenolic acids are gaining increasing attention in the scientific community. Humans ingest roughly 25 mg of phenolic acids everyday (24), most of which comes from the fruits, coffee, spices, and vegetables we eat regularly.

The research on a plant origin for a therapeutic medicinal material discovery or development is expensive and is a demanding effort [19]. It takes at least ten years and anywhere from $100-$360 million to bring a new medicine to market. Only around one-fourth of the 10,000 chemicals studied up until 1992 were actually usable as medicines. The National Cancer Institute evaluated 50,000 plant extracts for biological activity and discovered three biologically active compounds for treating human immunodeficiency virus and three biologically active compounds for anticancer activity [17]. They necessitated fundamental understanding of chemistry, biology, chemistry, pharmacology, and toxicity. The importance of each of these fields should not be downplayed in favor of the others. Organic chemistry, ethnography, biotechnology, agronomy, and basic pharmacological expertise all play significant roles in the development of any novel plant-based pharmaceutical [20]. Herbal teas or the creation of pharmaceutical powder tablets, tinctures, capsules, fluid extract, standard enhance, or crude extract are all viable options for therapeutic treatment (at home) when a medicinal plant is discovered. Medicinal drugs like ergotamine (via its precursor diogenin), digoxin, and quinine are all derived from plants that have active natural compounds that may be extracted and purified by an extraction procedure [21].

Identification of Plants

Prof. Tahira Israr, head of the Botany Department at ICAR's Pusa Campus in New Delhi, helped confirm the identification of Ginger and Cinnamon as the therapeutic plants.

Drying and Grinding of the Plant

The gathered plants were washed and then chopped up using knives and scissors. To facilitate drying and to shield them from environmental contaminants and dust, they were placed in a shady area. The drying process took roughly three weeks (with no exposure to light) inside a dark room. After

completely drying the plants, obtain uniform-sized powder and ensure to expand the surface area for better extraction process.

Extraction

***Maceration*:** The drug's leaves, stem bark, or root bark are finely pulverized and placed in a container for the extraction process. The menstruum is then poured on top until the drug is completely covered. After three days, seal the jar and set it aside. To achieve thorough extraction, the contents are mixed at regular intervals and, if placed in a bottle, shaken at regular intervals. Filtration or decantation is used to separate the micelle from the marc at the end of the extraction process. The micelle is then evaporated in an oven or on top of a water bath to isolate it from the menstruum. This is an easy and effective way to deal with thermosensitive plant matter.

Phenolic Extractions

The phenolic compounds were isolated using the methodology described by Chu et al. [40]. To isolate the free phenolics from the ethanol fraction, we filtered the mixture through Whatman filter paper and evaporated the mixture in a rotary evaporator at 45 degrees Celsius. The resulting dry extract was lyophilized and kept at six degrees below zero. While this was going on, the free phenolics and ethyl acetate fraction residue was hydrolyzed with 15 mL of NaOH (M = 2) at room temperature (22 6°C), the pH was adjusted to 2.1 0.1 with HCl, and the mixture was stirred constantly for 45 minutes to extract the bound phenolics. After that, we dried and evaporated (at temperatures below 45 degrees Celsius) the ethyl acetate and stored it for later use.

Determination of Total Phenolics

Singleton et al. provided an example of how the phenolic content was calculated. First, extracts were placed in an incubation bath containing Na2CO3 (8%, 2 mL) and Folin-Ciocalteu's reagent (10%, 3 mL) for oxidation and neutralization during a 30-minute period at 40 degrees Celsius. Finally, a UV-spectrophotometer was used to measure the absorbance at 765 nm. For certain measurements, gallic acid served as the gold standard.

Determination of Reducing Power

Oyaizu provides an example of how the reducing power can be calculated using the reducing power of a solution of FeCl3. To begin, 3 milliliters of K3[Fe(CN)6] (Potassium Ferricyanide) 1%, 3 milliliters of Na2HPO4 (Sodium Phosphate) M = 200 mM, and 3 milliliters of samples were combined and incubated at 40 degrees Celsius for 20 minutes. Second, 3 milliliters of trichloroacetic acid (TCA) (C2HCl3O2) were added, and the resulting mixture was centrifuged at 2600 revolutions per minute for 10 minutes to provide 5 milliliters of clear supernatant containing 0.1% ferric chloride (FeCl3) in distilled water. Finally, a UV-spectrophotometer was used to measure the absorbance at 710 nm. Ascorbic acid was utilized as the reference for certain numbers.

Invitro antioxidant

DPPH Assay

The DPPH free radical is a dark purple, extremely stable organic nitrogen radical. The color of a DPPH solution changes from purple to the yellow of the matching hydrazine when it is combined with an antioxidant. Antioxidants' ability to inhibit DPPH oxidation can be measured by their ability to lessen the radical's absorbance between 515 and 528 nanometers. All substances are tested at the same dose of antioxidants (IC50 or % scavenging of DPPH; Brand-Williams et al., 1995; Sanchez-Mareno, 2002).

Preparation of DPPH solution

DPPH solution was prepared by taking 7.89 mg of DPPH using a chemical balance, dissolving with 100 ml 99.5% ethanol, and finally kept in dark for 2 hr

DPPH assay procedure

DPPH solution of 1,000 µl was added with 800 µl of Tris-HCl buffer (pH 7.4) in a testing tube. And then 200 µl of testing sample solution was added and mixed quickly. The solution was kept at room temperature for 30 min. The absorbance of the solution at 517 nm was recorded. A mixed solution with 1,200 µl of ethanol and 800 µl of TrisHCl buffer (pH 7.4) was used as the blank. The inhibition ratio (%) was obtained from the following equation:

$$\textbf{Inhibition ratio (\%)} = (A1 - A2) \times 100/A1$$

where A1 is the absorbance of the addition of ethanol instead of testing sample and A2 is the absorbance of testing sample solution.

Statistical Calculations

At the chosen significance level of = 0.05, we ran one- and two-way ANOVA tests (SAS 9.2 2008 model) and multiple T-Tukey tests on the data we collected. An analysis of variance model with main effects and interactions of the researched components was utilized, and the comprehensive analysis focused mostly on the main effects and two-way interactions. T-Tukey's multiple comparison tests provide extensive comparative assessments of means by identifying statistically homogenous groupings of the mean (homogeneous groups), identified with the use of the least significant differences (LSDs). The p-values, which are estimated probabilities associated with the F-test functions used (F-Snedecor or Fisher-Snedecor), are among the most important aspects of analysis of variance and are included in the results tables. By comparing the generated p values with the generally recognized significance levels (0.05), the extent and significance of the influence of the studied factors on the differentiation of the outcomes of the analyzed variables may be determined.

3. Results and Discussion

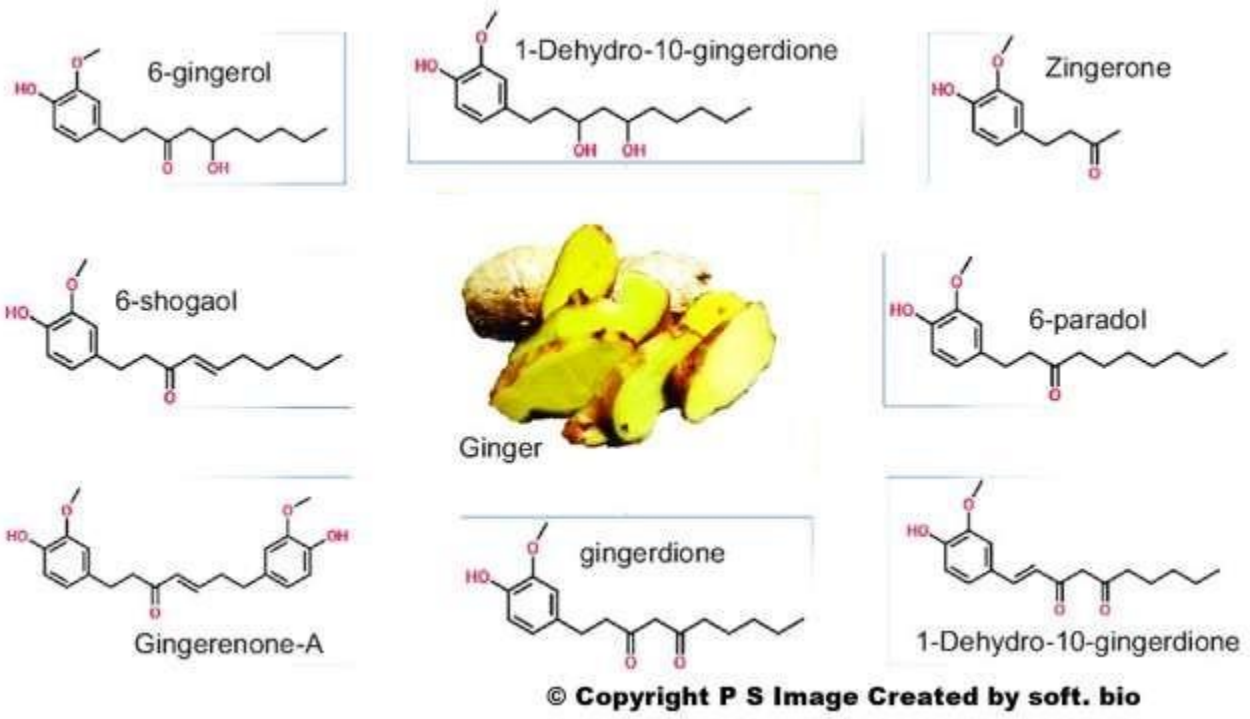


Fig: 1 Ginger Bioctive compound

Table: 1 Chemical Element Present in Poly herbal formulation

Element spectral line	Minimum found	Maximum found	NIST 1515 Found	LOD	LOQ
P (213.618 nm)	**5.19**±0.08	**1245**±22	**1583**±2	**0.03**	**0.08**
S (182.034 nm)	**7.45**±0.04	**1687**±14	**1078**±4	**0.04**	**0.14**
Mg (250.280 nm)	**479**±3	**1452**±47	**2835**±7	**0.06**	**0.17**
Ca (422.673 nm)	**5300**±20	**7415**±47	**13745**±175	**0.67**	**1.01**
K (766.490 nm)	**3874**±45	**10.5**±0.4	**14078**±0.04	**0.32**	**0.12**
Cu (324.754 nm)	**2.55**±0.03	**24**±02	**12**±2	**0.04**	**0.12**
Zn (213.856 nm)	**5.4**±0.1	**451**±12	**3150**±0.06	**0.02**	**0.010**
B (249.773nm)	**9.3**±1.4	**45**±0.3	**61.4**±0.4	**0.1**	**0.47**
Fe (259.940 nm)	**18.0**±4.6	**356**±0.4	**5542**±14	**0.60**	**0.16**
Al (247.784 nm)	**28.5**±7.6	**78**±06	**478**±5	**0.9**	**0.45**
Mn(345.457 nm)	**147**±0.14	**245**±4.5	**44**±5	**0.78**	**0.18**

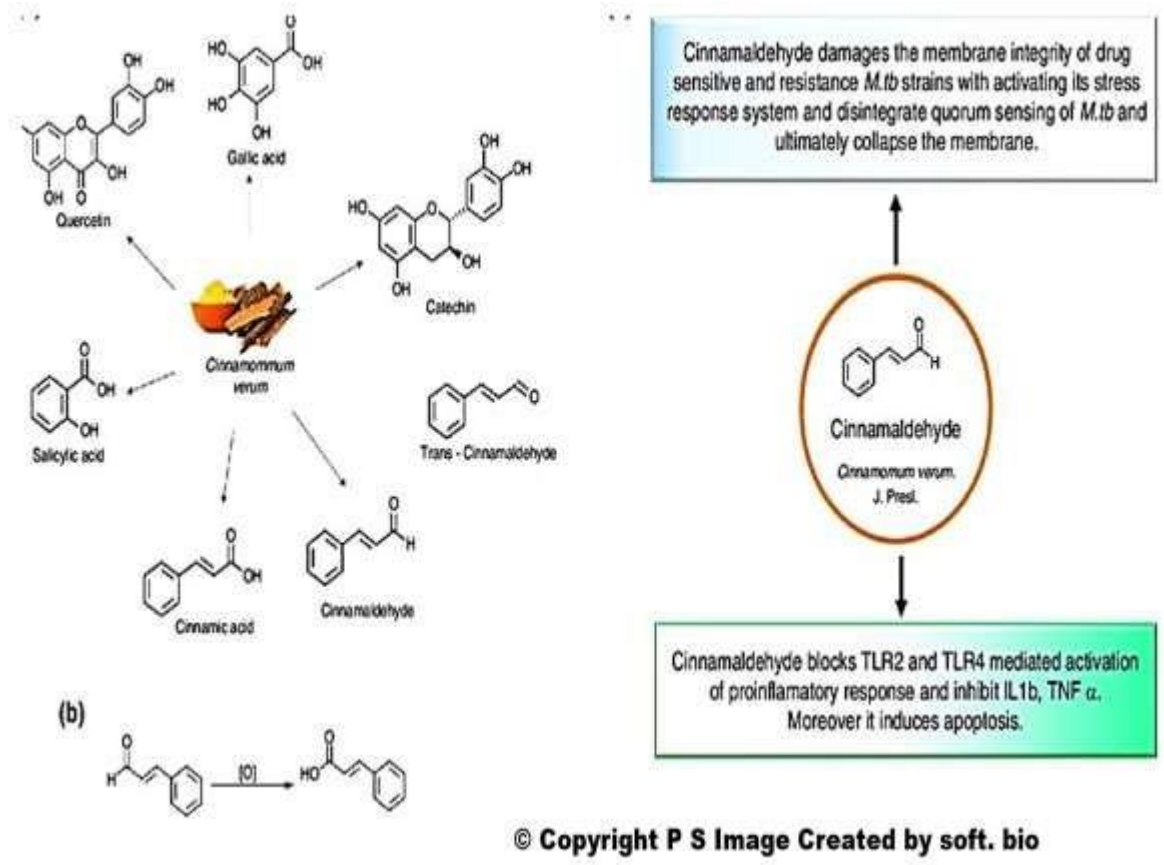


Table 2 Total Phenolic and flavonoids of Giner and Cinnamon extraction rate in methanolic extract

Specification	Symbol	T1	T2	T3	Mean	LSD 0.05
Phenolic Compound	TPC	22.78	25.14	25.42	24.89±1.47	1.14
Flavonoid Content	TPC	19.23	17.98	18.77	18.77±0.78	0.97
Rate of Methanol Extract	R%	15.61	14.58	15.44	15.47±0.57	0.46

The total amount of phenols and flavonoids found in this experiment are shown in Table 2. For starters, it's clear from this information that the plant is loaded with healthy phenolic chemicals and flavonoids. In this work, we used a non-exhaustive cold maceration extraction method and produced sufficient yields to accomplish our study target, with the rate of methanolic extraction for the whole Parts of Cinnamon & Ginger coming in at around 15.17%.

According to the data we have on phenolic compounds, flavonoids, and their extraction rates, they are extremely stable (V = 3.55–6.06%, respectively) throughout time. table 3. When we use the median to categorize our data, we can easily distinguish between findings that are below the median and those that are above it. In other words, if half of our results were lower than the median value and the other half were higher than the median value, then we know that the median number represents the middle value. Phenolic compounds had an SD of 1.48, while flavonoids had an SD of 0.72. The skewedness of the results was evaluated. It reveals the dispersion of values around the mean for a specified variable. Whether most of the data points are clustered around the mean, to the left of the mean, or to the right of the mean. All asymmetry was determined to be left-handed. Our set of observations has a certain range, which we can infer from the difference between the highest and lowest values in our data set (i.e., the range). The gap between the highest and minimum value was minor, which indicates the stability of these properties. To make a fair evaluation, we can use a relative measure of variability called the coefficient of variability to evaluate the qualities under scrutiny. The homogeneity of the Ginger and Cinnamon extract population is supported by the low value of the coefficient of variation of phenolic compounds and flavonoids.

Table 3 Total Phenolic and total Flavonoids of Cinnamon and ginger extract in methanol extract

Specification	TPC	TFC	R%
Mean	24.49	18.95	14.12
Median	25.15	18.76	15.45
Standard deviation	1.47	0.74	0.45
Slowness	-1.61	-1.45	-1.55

Range	2.74	1.47	1.05
Maximum	22.79	17.48	14.78
Minimum	25.65	19.35	15.45

Biological Activity

The total amount of phenols and flavonoids found in this experiment are shown in Table 2. For starters, it's clear from this information that the plant is loaded with healthy phenolic chemicals and flavonoids. In this work, we used a non-exhaustive cold maceration extraction method and produced sufficient yields to accomplish our study target, with the rate of methanolic extraction for the whole Parts of Cinnamon & Ginger coming in at around 15.17%.

According to the data we have on phenolic compounds, flavonoids, and their extraction rates, they are extremely stable (V = 3.55–6.06%, respectively) throughout time. table 3. When we use the median to categorize our data, we can easily distinguish between findings that are below the median and those that are above it. In other words, if half of our results were lower than the median value and the other half were higher than the median value, then we know that the median number represents the middle value. Phenolic compounds had an SD of 1.48, while flavonoids had an SD of 0.72. The skewedness of the results was evaluated. It reveals the dispersion of values around the mean for a specified variable. Whether most of the data points are clustered around the mean, to the left of the mean, or to the right of the mean. All asymmetry was determined to be left-handed. Our set of observations has a certain range, which we can infer from the difference between the highest and lowest values in our data set (i.e., the range). The gap between the highest and minimum value was minor, which indicates the stability of these properties. To make a fair evaluation, we can use a relative measure of variability called the coefficient of variability to evaluate the qualities under scrutiny. The homogeneity of the Ginger and Cinnamon extract population is supported by the low value of the coefficient of variation of phenolic compounds and flavonoids.

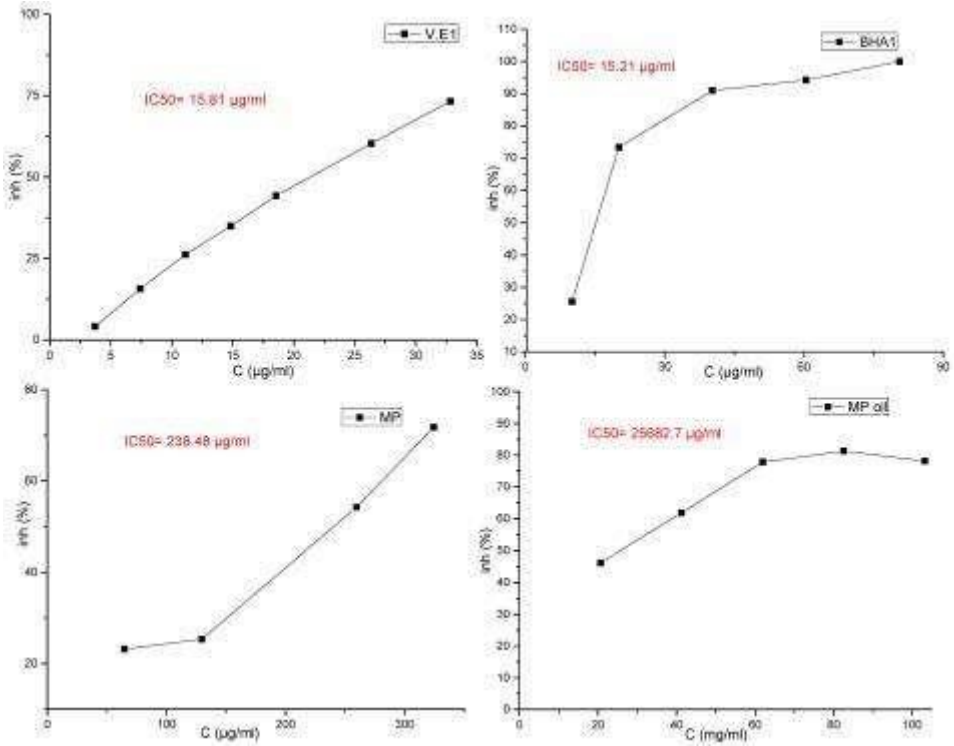


Fig: 3 Antioxidant power of essential oil and methanolic extract of poly herbal study in vitro via the inhibition of DPPH

Table: 4 Antioxidant power of essential oil and methanolic extract of poly herbl study in vitro via the inhibition of DPPH

Specification	Symbol	DPPH IC_{50}
Essential oil	MP Oil	25.6827
Methanolic extract	MP	15.65
Compose of reference	Butylhydroxyanisol	15.50

4. Conclusion

This study covers the phytochemical analysis, mineral elements, biological potentials, and effect of extraction methods on the chemical compositions of essential oil of whole plant sections of the plant Ginger and cinnamon a wild-growing plant in the Algerian high plains. Hydrodistillation, steam distillation, and microwave distillation were all used to separate the essential oils. In terms of extraction yield and main chemical quantification, the HD and SD excelled, while the MAD required only half the time, 30 minutes. Minerals, especially micro- and macronutrients, play a crucial role in human health since they are required for a wide range of metabolic processes. Ginger and cinnamon were found to contain high concentrations of calcium (31,875 mg/kg), potassium (14,216 mg/kg), iron (1604 mg/kg), sodium (10,790 mg/kg), magnesium (71 mg/kg), manganese (71.2 mg/kg), zinc (44.5 mg/kg), and other minerals essential to human health. The plant's unique pharmaceutical and therapeutic qualities can be attributed to the fact that its level of potentially harmful components was far lower than the toxicological reference values compared to the tolerance limits set by the World Health Organization. The data showed that compared to cinnamon essential oil, Ginger methanol extract has much better antioxidant activity. However, it's not quite as high as the BHT and V.E. positive controls.

Author Contribution:

Writing and drafting: Jytosna Upadhyay, Mariam Aysha A, K. Aishwarya, V.S Subashini, M. Sangeetha **All Software & Figure Conceptualization:** Roshan Kumar, Prachi Sood **Original Draft Preparation**: Farha Naaz **Final Review and editing:** Roshan Kumar **Investigation formal Analysis:** Prachi Sood. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest: None

Funding Agency: There is no any funding Agency.

References:

1. Kaur, R. P., Vasudeva, K., Kumar, R., & Munshi, A. (2018). Role of p53 gene in breast cancer: focus on mutation spectrum and therapeutic strategies. *Current pharmaceutical design*, *24*(30), 3566-3575.
2. Awuchi, C. G., Amagwula, I. O., Priya, P., Kumar, R., Yezdani, U., & Khan, M. G. (2020). Aflatoxins in foods and feeds: A review on health implications, detection, and control. *Bull. Environ. Pharmacol. Life Sci*, *9*, 149-155.
3. Gérard, A., Woolfe, A., Mottet, G., Reichen, M., Castrillon, C., Menrath, V., ... & Brenan, C. (2020). High-throughput single-cell activity-based screening and sequencing of antibodies using droplet microfluidics. *Nature biotechnology*, *38*(6), 715-721.
4. Mishra, A., Singh, Y., Singh, R., Kumar, R., Shukla, S., Kumar, R., ... & Pol, S. L. (2022). Ethano-pharmacology activity & Antioxidant activity of Centella asiatica Plant Parts. *Neuroquantology*, *20*(11), 7562-2.
5. Afzaal, M., Saeed, F., Ateeq, H., Akhtar, M. N., Imran, A., Ahmed, A., ... & Awuchi, C. G. (2023). Probiotics encapsulated gastroprotective cross-linked microgels: Enhanced viability under stressed conditions with dried apple carrier. *Food Science & Nutrition*, *11*(2), 817-827.
6. Chaudhary, H., Sagar, S., Kumar, R., Bisht, V., & Butola, K. (2022). Herbal Essential Oil use as Ulcer Protective Activity: A Systematic Review. *Journal for Research in Applied Sciences and Biotechnology*, *1*(5), 86-101.
7. Patel, R. K., Gangwar, D., Gupta, H., Sharma, N., & Kumar, R. (2023). Plants Alkaloids Based Compound as Therapeutic Potential for Neurodegenerative. *Journal for Research in Applied Sciences and Biotechnology*, *2*(2), 14-26.
8. Keshri, S., Kumar, R., Kumar, D., Singhal, T., Giri, S., Sharma, I., & Vatsha, P. (2022). Insights Of Artificial Intelligence In Brain Disorder With Evidence Of Opportunity And Future Challenges. *Journal of Pharmaceutical Negative Results*, 10853-10867.
9. Butola, K., Bisht, V., & Kumar, R. (2023). Recent Approaches of Ocular Disease and Its Herbal Product Treatment: An Updates. *Journal for Research in Applied Sciences and Biotechnology*, *2*(2), 102-114.
10. Raj, R., Kumar, A., Sood, P., Kumar, R., & Rana, V. (2023). Randomized Phase III Trial Comparing Epirubicin/Doxorubicin Plus Docetaxel and Epirubicin/Doxorubicin Plus Paclitaxel as First Line Treatment in Women with Advanced Breast Cancer. *Journal for Research in Applied Sciences and Biotechnology*, *2*(3), 55-63.
11. Kumar, R. (2023). Investigation of In-Vitro Method of Antiulcer Activity. *Journal for Research in Applied Sciences and Biotechnology*, *2*(1), 264-267.
12. Kumar, R., Sood, P., Nirala, R. K., Ade, R. P., & Sonaji, A. V. (2023). Uses of AI in Field of Radiology- What is State of Doctor & Pateints Communication in Different Disease for Diagnosis Purpose. *Journal for Research in Applied Sciences and Biotechnology*, *2*(5), 51–60. https://doi.org/10.55544/jrasb.2.5.9
13. Kumar, R., Sood, P., Nirala, R. K., Christian, A., Nyarko, R. O., Boateng, E. A., Asante-Duku, F., & Orlando, V. K. (2023). Plant & its Bioactive Components Uses in Cardio-Potential Diseases: A Sectional Study for Different Herbs. *Journal for Research in Applied Sciences and Biotechnology*, *2*(5), 61–71. https://doi.org/10.55544/jrasb.2.5.10

14. Wongkuna, S., Kumar, R., Ghimire, S., Drescher, S. M., Maji, A., Ambat, A., ... & Scaria, J. (2022). Identification of a microbial sub-community from the feral chicken gut that reduces Salmonella colonization and improves gut health in a gnotobiotic chicken model. *bioRxiv*, 2022-07.
15. Keshri, S., Kumar, R., Kumar, D., Singhal, T., Giri, S., Sharma, I., & Vatsha, P. (2022). Insights Of Artificial Intelligence In Brain Disorder With Evidence Of Opportunity And Future Challenges. *Journal of Pharmaceutical Negative Results*, 10853-10867.