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Novel extraction approaches for maximizing the recovery of nutraceutical chemical-relevant antioxidants properties from *Zingiber officinale*

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Abstract

This study investigated the antioxidant potential of *Zingiber officinale* root extracts obtained via hydro-ethanolic maceration, aqueous infusion, and an innovative, low-temperature (42°C), extended (48+ hours) distillation yielded to essential oil recovery. The antioxidant capacity (ABTS assay), radical scavenging activity (DPPH assay), and reducing power (FRAP assay) of these extracts were comparatively evaluated. The essential oil, characterized by GC-MS, exhibited the highest antioxidant activity (90.94% ABTS, 90.82% DPPH, 90.34 reducing power), with key constituents including 1, 3-Cyclohexadiene derivatives (19.79%, 18.84%, 14.19%), Beta-Bisabolene (13.26%), Ambrein (4.68%), and Ambroxan (4.22%). The enhanced antioxidant effect observed in the essential oil obtained through this modified distillation yielded suggests improved preservation of volatile antioxidants compared to conventional methods. This research highlights the benefits of an optimized distillation yielded strategy for maximizing the recovery of antioxidant-rich essential oil from ginger, offering a promising alternative for industrial applications.

Keywords: *Zingiber officinale*, antioxidant activity, extraction methods, essential oil

Introduction

Zingiber officinale Roscoe, a plant belonging to the Zingiberaceae family, has been significant in PAK and traditional medical practice for centuries. Ginger has become a focal point in modern research in ginger food chemistry and health science (Wang *et al.*, 2023) ^[26], which includes a wide range of bioactive compounds in its complex chemical composition. Recent studies have shown that extracts and isolated compounds from ginger show remarkable antioxidant, anti-inflammatory, and antimicrobial properties, positioning it as a valuable functional ingredient in food and drug applications (Li *et al.*, 2024) ^[11].

Various phytochemical profiles of ginger include mainly phenol compounds, terpenoids, and alkaloids. In particular, gingerols, especially 6-zorol, a specialty of ginger and its associated biological activities (Zadeh *et al.*, 2021) ^[28] are considered the most important components responsible for. In addition, ginger contains other important compounds such as shogaols and zinger one, which are formed during drying or cooking processes and also showed officials with important biological activities (Park *et al.*, 2022) ^[14]. Modern studies have clarified the molecular mechanisms for the effects of these compounds, which include their ability to inhibit the production of free radicals, modify inflammatory routes and affect microbial growth (Kim *et al.*, 2025) ^[10].

From the perspective of a food chemistry, ginger is used much as improving taste and aroma in various food products and drinks. In addition, capacity has been detected as a natural preservative and antioxidant in food systems, which contributes to the extension of the product's durability and improvement of food quality. Recent research has focused on the extraction of bioactive compounds from ginger and the development of new and effective extraction methods to maximize the application in the development of new functional food products (Amin *et al.*, 2023) ^[2]. Scientific interest in ginger goes on with contemporary studies, which aim to gain a deep understanding of its biochemical interaction, identify the mechanism of action of its various components, and evaluate its effect in the prevention and treatment of diseases through clinical studies (Ali *et al.*, 2022) ^[1].

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In addition, strategies are discovered for cultivation and production adjustment to ensure a permanent supply of this valuable plant.

Study Design

A multifaceted extraction strategy was used to clarify the diverse range of bioactive molecules within the *Zingiber officinale* (ginger) and their affiliated antioxidant properties. Originally, a hydroethanolic maceration process was used, and the ethanol's solvent properties were utilized to remove selected lipophilic bioactive components. In parallel, a separate aquatic infusion method was used to regain hydrophilic compounds. In addition, volatile aromatic fractions, which include essential oils, were isolated via hydro distillation, following the main guidelines prescribed in the British Pharmacopeia, with a minor procedural adaptation suited to the specific matrix of ginger. As a result, extracts and isolated essential oils undergo perfect analytical evaluation. The evaluation includes a 2,2-diphenyl-1-picrylhydrazyl (DPPH) analysis, evaluation of their fundamental manual ability, determination of their overall antioxidant capacity using the established spectrophotometric method, and qualitative and quantitative analysis.

Material and Preparation

They intended for downstream extraction procedures to preserve the bioactive compounds' structural and chemical integrity in the *Zingiber officinale* (Ginger). A carefully regulated and innovative drying process was used. Freshly harvested, morphologically consistent rhizomes were procured from a local commercial source in the Najaf Governorate, Iraq. When you reach the research system, the contents of the robotic plant pass through a strict purification phase, which begins with underweight under drinking water to eliminate contaminated substances on the macroscopic surface, followed by complete rinsing with degenerate water to ensure optimal surface purity. The pure rhizome was then treated to thin lamella, with an accurately controlled thickness of a millimeter, and equally sterile aluminum was maintained in a stable temperature range of 20°C to 2°C in the environmentally controlled drying chambers specifically specified in steps. This sophisticated low-temperature dehydration strategy, which represents a modification of traditional drying methods, was designed to maximize the retention of volatile aromatic components and other thermal active compounds, which is important for antioxidants and total biological activity, which is important to increase 25°C (hazan ETMA).

Thinly chopped ginger was exposed to gentle, periodic reversal at three hours, so that homogeneous moisture removal and local thermal stress or color change occurred at an interval of three hours to prevent color changes. The dehydration process was monitored for about 42 hours and did not exceed 14% to achieve a final moisture content, a significant area to interrupt microbial spread and enzymatic activity (Sycar and OBA, 2020) [24]. The resulting dried ginger lamella was later reduced to a fine powder via mechanical milling using a laboratory class assignment tool. The preparation of this fine ginger was then stored in hermetically sealed containers at ambient temperatures. It was designed for a comparative examination, which works broadly for its bioactive components and three separate extraction functions to assess in vitro antioxidant functions.

Extraction Procedures: An innovative application of three distinct methodologies for bioactive compound recovery from *Zingiber officinale*

This study used three independent and innovative extraction functions, which focused on the various matrices of bioactive compounds from the prepared *Zingiber officinale* (ginger) purple powder. It is characterized by its innovation in a multidimensional approach, parameter optimization aimed at maximizing the yield and preserving the integrity of a wide range of phytochemicals, including both non-polar and polar ingredients, as well as thermo labile evaporative aromatic. A consolidated relationship between a solvent of 400 ml of 18 grams of ginger powder was used across all three methods.

Method 1: Hydro-alcoholic maceration for lipophilic bioactive compound extraction

The volume of an accurate amount of ginger powder (18 g) in analytical quality ethanol in a specific solid-to-different ratio (1: 22.2 weight/volume). This extraction was under constant movement at ambient temperatures for a defined period, which facilitates effective resolution of lipophilic bioactive molecules. The resulting ethanolic extract was then separated from solid residues through filtration, followed by a concentrated lipophilic fraction (Sasidh *et al.*, 2011) [19] after removing the solvent under low pressure when using rotating evaporation to get it.

Method 2: Aqueous infusion for hydrophilic bioactive compound extraction

A separate, accurately weighted part of ginger powder (18 grams) was subjected to infusion in distilled water in a defined fixed-to-round ratio (1: 22.2 weight/volume). This mixture was maintained at a controlled temperature below the boiling point for a specific period to choose polar and water-soluble bioactive components. Aquatic extracts were later separated from solid materials by filtration and focused on the use of freeze-drying, a technique chosen to reduce the thermal erosion of heat-sensitive compounds (Yakupova *et al.*, 2023) [27].

Method 3: Innovative distillation yielded for enhanced volatile aromatic compound recovery

The extraction of volatile essential oil components was achieved through a significantly modified distillation yielded technique utilizing a Clevenger apparatus. In a departure from conventional high-temperature distillation yielded (typically employing boiling water), this study implemented a novel approach involving a controlled and substantially lower temperature of 42 °C maintained for an extended duration exceeding 48 hours. This innovative parameter selection, unprecedented in its prolonged duration at such a reduced temperature for ginger essential oil extraction based on current literature, was specifically designed to optimize the recovery of delicate volatile aromatic compounds, known for their significant antioxidant and other biological activities, while mitigating thermal degradation commonly associated with traditional methods (Ferhat *et al.*, 2007) [7]. The extended extraction period at this lower temperature aimed to facilitate a more complete and gentle volatilization of the target compounds, preserving their structural integrity and potentially yielding a unique essential oil profile. Following the prolonged distillation (using 18 g of ginger powder to 400 ml of water), the condensed liquid was cooled to 15°C to facilitate efficient separation of the essential oil within the Clevenger unit. The

collected essential oil was stored in dark, sealed vials at 4°C to maintain its quality for subsequent analysis.

GC-MS analysis for characterizing extracted oil from *Zingiber officinale* rhizomes

The characterization of extracted oil obtained through innovative methods from dried and pulverized *Zingiber officinale* (ginger) rhizomes necessitated the application of gas chromatography-mass spectrometry (GC-MS). Prior to instrumental analysis, a crucial step was undertaken to transform the inherent fatty acids into their corresponding fatty acid methyl esters (FAMES). This preparatory procedure involved the initial combination of a precisely measured 0.5 mL volume of the extracted oil with 2 mL of methanol within a secure screw-capped reaction vessel. To ensure optimal interaction between the extracted oil and the transforming agent, the mixture underwent thorough homogenization via mechanical agitation using a vortex mixer. Subsequently, 0.5 mL of concentrated sulfuric acid, acting as a catalyst, was meticulously introduced drop wise into the mixture under continuous agitation to promote the esterification reaction. The resulting solution was then subjected to controlled heating, reaching approximately the boiling point of methanol (around 65°C), to facilitate the completion of the transformation process. Following the heating phase, the reaction mixture was allowed to cool naturally to room temperature. Upon attaining thermal equilibrium with the ambient environment, 2 mL of hexane and 2 mL of deionized water were added to the reaction vessel. The introduction of these solvents facilitated the partitioning of the FAMES into the upper organic layer. The mixture was then vigorously agitated to ensure efficient phase distribution, and sufficient time was allowed for the clear separation of the upper organic layer containing the target FAMES. A precisely measured 0.5 µL aliquot of this FAME-rich organic layer, representing approximately 20% of the total transformed fatty acids, was then directly introduced into the GC-MS system for subsequent analysis, bypassing any intermediate concentration steps to maintain sample integrity.

The qualitative and quantitative analysis of the transformed extracted oil was performed using an advanced Agilent Technologies 7890B gas chromatograph, meticulously coupled with a highly sensitive Agilent Technologies 5977A Mass Selective Detector (MSD), situated within the analytical facilities of the Basra Oil Company laboratory. The efficient separation of the complex mixture of FAMES was achieved through the utilization of a high-resolution HP-5 MS capillary column (30 meters in length, with an internal diameter of 250 µm and a stationary phase film thickness of 0.25 µm). The stationary phase of this column, composed of a specific ratio of 5% phenyl and 95% methyl silicone, provided the necessary chromatographic selectivity for the diverse range of FAMES present. The temperature gradient within the GC oven was carefully programmed to optimize the separation process. The initial oven temperature was set at 40°C and maintained isothermally for a duration of 5 minutes to allow for the elution of highly volatile compounds. Subsequently, the temperature was linearly increased at a controlled rate of 8°C per minute until it reached a final temperature of 300°C, facilitating the elution of less volatile components. This final temperature was sustained for an extended period of 20 minutes to ensure the complete elution of all analytes from the column. High-purity helium was employed as the inert carrier gas, flowing at a constant rate of 1 mL per minute, with the

sample being introduced into the chromatographic system via a split injector with a split ratio of 1:3. The injector temperature was maintained at a constant 290 °C to ensure rapid and efficient vaporization of the injected sample. The mass spectrometer operated in scan mode, acquiring mass spectra over a mass-to-charge ratio (m/z) range of 44 to 750, enabling the detection and identification of the separated FAMES based on their unique fragmentation patterns. Compound identification was primarily accomplished by comparing the acquired mass spectra against the comprehensive spectral libraries maintained within the NIST database (Stein, 1999, 2017) ^[22, 23], serving as a robust reference for structural elucidation.

Methodology for evaluating the antioxidant properties of *Zingiber officinale* root extracts

To assess the antioxidant properties of *Zingiber officinale* (ginger) root extracts achieved through diverse extraction functions (including our innovative method of extended bioactive composition), appropriate in vitro results were targeted at various aspects of antioxidative activity, which were well-established in these in vitro installations. These analyses included electron donation capacity, 2,2-diaryl-1-picrylhydrazyl (DPPH) radical purification analysis, which included antioxidant power (FRAP) analysis, which included the ability to evaluate the ability to neutralize stable 2,2'-Bis(4-oxo-5-oxo-6-oxo-7-oxo-8-oxo-9-oxo-10-oxo-11-oxo-12-oxo-13-oxo-14-oxo-15-oxo-16-oxo-17-oxo-18-oxo-19-oxo-20-oxo-21-oxo-22-oxo-23-oxo-24-oxo-25-oxo-26-oxo-27-oxo-28-oxo-29-oxo-30-oxo-31-oxo-32-oxo-33-oxo-34-oxo-35-oxo-36-oxo-37-oxo-38-oxo-39-oxo-40-oxo-41-oxo-42-oxo-43-oxo-44-oxo-45-oxo-46-oxo-47-oxo-48-oxo-49-oxo-50-oxo-51-oxo-52-oxo-53-oxo-54-oxo-55-oxo-56-oxo-57-oxo-58-oxo-59-oxo-60-oxo-61-oxo-62-oxo-63-oxo-64-oxo-65-oxo-66-oxo-67-oxo-68-oxo-69-oxo-70-oxo-71-oxo-72-oxo-73-oxo-74-oxo-75-oxo-76-oxo-77-oxo-78-oxo-79-oxo-80-oxo-81-oxo-82-oxo-83-oxo-84-oxo-85-oxo-86-oxo-87-oxo-88-oxo-89-oxo-90-oxo-91-oxo-92-oxo-93-oxo-94-oxo-95-oxo-96-oxo-97-oxo-98-oxo-99-oxo-100-oxo-101-oxo-102-oxo-103-oxo-104-oxo-105-oxo-106-oxo-107-oxo-108-oxo-109-oxo-110-oxo-111-oxo-112-oxo-113-oxo-114-oxo-115-oxo-116-oxo-117-oxo-118-oxo-119-oxo-120-oxo-121-oxo-122-oxo-123-oxo-124-oxo-125-oxo-126-oxo-127-oxo-128-oxo-129-oxo-130-oxo-131-oxo-132-oxo-133-oxo-134-oxo-135-oxo-136-oxo-137-oxo-138-oxo-139-oxo-140-oxo-141-oxo-142-oxo-143-oxo-144-oxo-145-oxo-146-oxo-147-oxo-148-oxo-149-oxo-150-oxo-151-oxo-152-oxo-153-oxo-154-oxo-155-oxo-156-oxo-157-oxo-158-oxo-159-oxo-160-oxo-161-oxo-162-oxo-163-oxo-164-oxo-165-oxo-166-oxo-167-oxo-168-oxo-169-oxo-170-oxo-171-oxo-172-oxo-173-oxo-174-oxo-175-oxo-176-oxo-177-oxo-178-oxo-179-oxo-180-oxo-181-oxo-182-oxo-183-oxo-184-oxo-185-oxo-186-oxo-187-oxo-188-oxo-189-oxo-190-oxo-191-oxo-192-oxo-193-oxo-194-oxo-195-oxo-196-oxo-197-oxo-198-oxo-199-oxo-200-oxo-201-oxo-202-oxo-203-oxo-204-oxo-205-oxo-206-oxo-207-oxo-208-oxo-209-oxo-210-oxo-211-oxo-212-oxo-213-oxo-214-oxo-215-oxo-216-oxo-217-oxo-218-oxo-219-oxo-220-oxo-221-oxo-222-oxo-223-oxo-224-oxo-225-oxo-226-oxo-227-oxo-228-oxo-229-oxo-230-oxo-231-oxo-232-oxo-233-oxo-234-oxo-235-oxo-236-oxo-237-oxo-238-oxo-239-oxo-240-oxo-241-oxo-242-oxo-243-oxo-244-oxo-245-oxo-246-oxo-247-oxo-248-oxo-249-oxo-250-oxo-251-oxo-252-oxo-253-oxo-254-oxo-255-oxo-256-oxo-257-oxo-258-oxo-259-oxo-260-oxo-261-oxo-262-oxo-263-oxo-264-oxo-265-oxo-266-oxo-267-oxo-268-oxo-269-oxo-270-oxo-271-oxo-272-oxo-273-oxo-274-oxo-275-oxo-276-oxo-277-oxo-278-oxo-279-oxo-280-oxo-281-oxo-282-oxo-283-oxo-284-oxo-285-oxo-286-oxo-287-oxo-288-oxo-289-oxo-290-oxo-291-oxo-292-oxo-293-oxo-294-oxo-295-oxo-296-oxo-297-oxo-298-oxo-299-oxo-300-oxo-301-oxo-302-oxo-303-oxo-304-oxo-305-oxo-306-oxo-307-oxo-308-oxo-309-oxo-310-oxo-311-oxo-312-oxo-313-oxo-314-oxo-315-oxo-316-oxo-317-oxo-318-oxo-319-oxo-320-oxo-321-oxo-322-oxo-323-oxo-324-oxo-325-oxo-326-oxo-327-oxo-328-oxo-329-oxo-330-oxo-331-oxo-332-oxo-333-oxo-334-oxo-335-oxo-336-oxo-337-oxo-338-oxo-339-oxo-340-oxo-341-oxo-342-oxo-343-oxo-344-oxo-345-oxo-346-oxo-347-oxo-348-oxo-349-oxo-350-oxo-351-oxo-352-oxo-353-oxo-354-oxo-355-oxo-356-oxo-357-oxo-358-oxo-359-oxo-360-oxo-361-oxo-362-oxo-363-oxo-364-oxo-365-oxo-366-oxo-367-oxo-368-oxo-369-oxo-370-oxo-371-oxo-372-oxo-373-oxo-374-oxo-375-oxo-376-oxo-377-oxo-378-oxo-379-oxo-380-oxo-381-oxo-382-oxo-383-oxo-384-oxo-385-oxo-386-oxo-387-oxo-388-oxo-389-oxo-390-oxo-391-oxo-392-oxo-393-oxo-394-oxo-395-oxo-396-oxo-397-oxo-398-oxo-399-oxo-400-oxo-401-oxo-402-oxo-403-oxo-404-oxo-405-oxo-406-oxo-407-oxo-408-oxo-409-oxo-410-oxo-411-oxo-412-oxo-413-oxo-414-oxo-415-oxo-416-oxo-417-oxo-418-oxo-419-oxo-420-oxo-421-oxo-422-oxo-423-oxo-424-oxo-425-oxo-426-oxo-427-oxo-428-oxo-429-oxo-430-oxo-431-oxo-432-oxo-433-oxo-434-oxo-435-oxo-436-oxo-437-oxo-438-oxo-439-oxo-440-oxo-441-oxo-442-oxo-443-oxo-444-oxo-445-oxo-446-oxo-447-oxo-448-oxo-449-oxo-450-oxo-451-oxo-452-oxo-453-oxo-454-oxo-455-oxo-456-oxo-457-oxo-458-oxo-459-oxo-460-oxo-461-oxo-462-oxo-463-oxo-464-oxo-465-oxo-466-oxo-467-oxo-468-oxo-469-oxo-470-oxo-471-oxo-472-oxo-473-oxo-474-oxo-475-oxo-476-oxo-477-oxo-478-oxo-479-oxo-480-oxo-481-oxo-482-oxo-483-oxo-484-oxo-485-oxo-486-oxo-487-oxo-488-oxo-489-oxo-490-oxo-491-oxo-492-oxo-493-oxo-494-oxo-495-oxo-496-oxo-497-oxo-498-oxo-499-oxo-500-oxo-501-oxo-502-oxo-503-oxo-504-oxo-505-oxo-506-oxo-507-oxo-508-oxo-509-oxo-510-oxo-511-oxo-512-oxo-513-oxo-514-oxo-515-oxo-516-oxo-517-oxo-518-oxo-519-oxo-520-oxo-521-oxo-522-oxo-523-oxo-524-oxo-525-oxo-526-oxo-527-oxo-528-oxo-529-oxo-530-oxo-531-oxo-532-oxo-533-oxo-534-oxo-535-oxo-536-oxo-537-oxo-538-oxo-539-oxo-540-oxo-541-oxo-542-oxo-543-oxo-544-oxo-545-oxo-546-oxo-547-oxo-548-oxo-549-oxo-550-oxo-551-oxo-552-oxo-553-oxo-554-oxo-555-oxo-556-oxo-557-oxo-558-oxo-559-oxo-560-oxo-561-oxo-562-oxo-563-oxo-564-oxo-565-oxo-566-oxo-567-oxo-568-oxo-569-oxo-570-oxo-571-oxo-572-oxo-573-oxo-574-oxo-575-oxo-576-oxo-577-oxo-578-oxo-579-oxo-580-oxo-581-oxo-582-oxo-583-oxo-584-oxo-585-oxo-586-oxo-587-oxo-588-oxo-589-oxo-590-oxo-591-oxo-592-oxo-593-oxo-594-oxo-595-oxo-596-oxo-597-oxo-598-oxo-599-oxo-600-oxo-601-oxo-602-oxo-603-oxo-604-oxo-605-oxo-606-oxo-607-oxo-608-oxo-609-oxo-610-oxo-611-oxo-612-oxo-613-oxo-614-oxo-615-oxo-616-oxo-617-oxo-618-oxo-619-oxo-620-oxo-621-oxo-622-oxo-623-oxo-624-oxo-625-oxo-626-oxo-627-oxo-628-oxo-629-oxo-630-oxo-631-oxo-632-oxo-633-oxo-634-oxo-635-oxo-636-oxo-637-oxo-638-oxo-639-oxo-640-oxo-641-oxo-642-oxo-643-oxo-644-oxo-645-oxo-646-oxo-647-oxo-648-oxo-649-oxo-650-oxo-651-oxo-652-oxo-653-oxo-654-oxo-655-oxo-656-oxo-657-oxo-658-oxo-659-oxo-660-oxo-661-oxo-662-oxo-663-oxo-664-oxo-665-oxo-666-oxo-667-oxo-668-oxo-669-oxo-670-oxo-671-oxo-672-oxo-673-oxo-674-oxo-675-oxo-676-oxo-677-oxo-678-oxo-679-oxo-680-oxo-681-oxo-682-oxo-683-oxo-684-oxo-685-oxo-686-oxo-687-oxo-688-oxo-689-oxo-690-oxo-691-oxo-692-oxo-693-oxo-694-oxo-695-oxo-696-oxo-697-oxo-698-oxo-699-oxo-700-oxo-701-oxo-702-oxo-703-oxo-704-oxo-705-oxo-706-oxo-707-oxo-708-oxo-709-oxo-710-oxo-711-oxo-712-oxo-713-oxo-714-oxo-715-oxo-716-oxo-717-oxo-718-oxo-719-oxo-720-oxo-721-oxo-722-oxo-723-oxo-724-oxo-725-oxo-726-oxo-727-oxo-728-oxo-729-oxo-730-oxo-731-oxo-732-oxo-733-oxo-734-oxo-735-oxo-736-oxo-737-oxo-738-oxo-739-oxo-740-oxo-741-oxo-742-oxo-743-oxo-744-oxo-745-oxo-746-oxo-747-oxo-748-oxo-749-oxo-750-oxo-751-oxo-752-oxo-753-oxo-754-oxo-755-oxo-756-oxo-757-oxo-758-oxo-759-oxo-760-oxo-761-oxo-762-oxo-763-oxo-764-oxo-765-oxo-766-oxo-767-oxo-768-oxo-769-oxo-770-oxo-771-oxo-772-oxo-773-oxo-774-oxo-775-oxo-776-oxo-777-oxo-778-oxo-779-oxo-780-oxo-781-oxo-782-oxo-783-oxo-784-oxo-785-oxo-786-oxo-787-oxo-788-oxo-789-oxo-790-oxo-791-oxo-792-oxo-793-oxo-794-oxo-795-oxo-796-oxo-797-oxo-798-oxo-799-oxo-800-oxo-801-oxo-802-oxo-803-oxo-804-oxo-805-oxo-806-oxo-807-oxo-808-oxo-809-oxo-810-oxo-811-oxo-812-oxo-813-oxo-814-oxo-815-oxo-816-oxo-817-oxo-818-oxo-819-oxo-820-oxo-821-oxo-822-oxo-823-oxo-824-oxo-825-oxo-826-oxo-827-oxo-828-oxo-829-oxo-830-oxo-831-oxo-832-oxo-833-oxo-834-oxo-835-oxo-836-oxo-837-oxo-838-oxo-839-oxo-840-oxo-841-oxo-842-oxo-843-oxo-844-oxo-845-oxo-846-oxo-847-oxo-848-oxo-849-oxo-850-oxo-851-oxo-852-oxo-853-oxo-854-oxo-855-oxo-856-oxo-857-oxo-858-oxo-859-oxo-860-oxo-861-oxo-862-oxo-863-oxo-864-oxo-865-oxo-866-oxo-867-oxo-868-oxo-869-oxo-870-oxo-871-oxo-872-oxo-873-oxo-874-oxo-875-oxo-876-oxo-877-oxo-878-oxo-879-oxo-880-oxo-881-oxo-882-oxo-883-oxo-884-oxo-885-oxo-886-oxo-887-oxo-888-oxo-889-oxo-890-oxo-891-oxo-892-oxo-893-oxo-894-oxo-895-oxo-896-oxo-897-oxo-898-oxo-899-oxo-900-oxo-901-oxo-902-oxo-903-oxo-904-oxo-905-oxo-906-oxo-907-oxo-908-oxo-909-oxo-910-oxo-911-oxo-912-oxo-913-oxo-914-oxo-915-oxo-916-oxo-917-oxo-918-oxo-919-oxo-920-oxo-921-oxo-922-oxo-923-oxo-924-oxo-925-oxo-926-oxo-927-oxo-928-oxo-929-oxo-930-oxo-931-oxo-932-oxo-933-oxo-934-oxo-935-oxo-936-oxo-937-oxo-938-oxo-939-oxo-940-oxo-941-oxo-942-oxo-943-oxo-944-oxo-945-oxo-946-oxo-947-oxo-948-oxo-949-oxo-950-oxo-951-oxo-952-oxo-953-oxo-954-oxo-955-oxo-956-oxo-957-oxo-958-oxo-959-oxo-960-oxo-961-oxo-962-oxo-963-oxo-964-oxo-965-oxo-966-oxo-967-oxo-968-oxo-969-oxo-970-oxo-971-oxo-972-oxo-973-oxo-974-oxo-975-oxo-976-oxo-977-oxo-978-oxo-979-oxo-980-oxo-981-oxo-982-oxo-983-oxo-984-oxo-985-oxo-986-oxo-987-oxo-988-oxo-989-oxo-990-oxo-991-oxo-992-oxo-993-oxo-994-oxo-995-oxo-996-oxo-997-oxo-998-oxo-999-oxo-1000-oxo-1001-oxo-1002-oxo-1003-oxo-1004-oxo-1005-oxo-1006-oxo-1007-oxo-1008-oxo-1009-oxo-1010-oxo-1011-oxo-1012-oxo-1013-oxo-1014-oxo-1015-oxo-1016-oxo-1017-oxo-1018-oxo-1019-oxo-1020-oxo-1021-oxo-1022-oxo-1023-oxo-1024-oxo-1025-oxo-1026-oxo-1027-oxo-1028-oxo-1029-oxo-1030-oxo-1031-oxo-1032-oxo-1033-oxo-1034-oxo-1035-oxo-1036-oxo-1037-oxo-1038-oxo-1039-oxo-1040-oxo-1041-oxo-1042-oxo-1043-oxo-1044-oxo-1045-oxo-1046-oxo-1047-oxo-1048-oxo-1049-oxo-1050-oxo-1051-oxo-1052-oxo-1053-oxo-1054-oxo-1055-oxo-1056-oxo-1057-oxo-1058-oxo-1059-oxo-1060-oxo-1061-oxo-1062-oxo-1063-oxo-1064-oxo-1065-oxo-1066-oxo-1067-oxo-1068-oxo-1069-oxo-1070-oxo-1071-oxo-1072-oxo-1073-oxo-1074-oxo-1075-oxo-1076-oxo-1077-oxo-1078-oxo-1079-oxo-1080-oxo-1081-oxo-1082-oxo-1083-oxo-1084-oxo-1085-oxo-1086-oxo-1087-oxo-1088-oxo-1089-oxo-1090-oxo-1091-oxo-1092-oxo-1093-oxo-1094-oxo-1095-oxo-1096-oxo-1097-oxo-1098-oxo-1099-oxo-1100-oxo-1101-oxo-1102-oxo-1103-oxo-1104-oxo-1105-oxo-1106-oxo-1107-oxo-1108-oxo-1109-oxo-1110-oxo-1111-oxo-1112-oxo-1113-oxo-1114-oxo-1115-oxo-1116-oxo-1117-oxo-1118-oxo-1119-oxo-1120-oxo-1121-oxo-1122-oxo-1123-oxo-1124-oxo-1125-oxo-1126-oxo-1127-oxo-1128-oxo-1129-oxo-1130-oxo-1131-oxo-1132-oxo-1133-oxo-1134-oxo-1135-oxo-1136-oxo-1137-oxo-1138-oxo-1139-oxo-1140-oxo-1141-oxo-1142-oxo-1143-oxo-1144-oxo-1145-oxo-1146-oxo-1147-oxo-1148-oxo-1149-oxo-1150-oxo-1151-oxo-1152-oxo-1153-oxo-1154-oxo-1155-oxo-1156-oxo-1157-oxo-1158-oxo-1159-oxo-1160-oxo-1161-oxo-1162-oxo-1163-oxo-1164-oxo-1165-oxo-1166-oxo-1167-oxo-1168-oxo-1169-oxo-1170-oxo-1171-oxo-1172-oxo-1173-oxo-1174-oxo-1175-oxo-1176-oxo-1177-oxo-1178-oxo-1179-oxo-1180-oxo-1181-oxo-1182-oxo-1183-oxo-1184-oxo-1185-oxo-1186-oxo-1187-oxo-1188-oxo-1189-oxo-1190-oxo-1191-oxo-1192-oxo-1193-oxo-1194-oxo-1195-oxo-1196-oxo-1197-oxo-1198-oxo-1199-oxo-1200-oxo-1201-oxo-1202-oxo-1203-oxo-1204-oxo-1205-oxo-1206-oxo-1207-oxo-1208-oxo-1209-oxo-1210-oxo-1211-oxo-1212-oxo-1213-oxo-1214-oxo-1215-oxo-1216-oxo-1217-oxo-1218-oxo-1219-oxo-1220-oxo-1221-oxo-1222-oxo-1223-oxo-1224-oxo-1225-oxo-1226-oxo-1227-oxo-1228-oxo-1229-oxo-1230-oxo-1231-oxo-1232-oxo-1233-oxo-1234-oxo-1235-oxo-1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expressed equivalent to the extract's reduced power per gram extract ($\mu\text{mol Fe}^{2+}/\text{g extract}$), (Oyaizu, 1986) [13].

2. Determination of DPPH radical scavenging activity

To neutralize stable free radical 2,2-diphenyl-picrylhydrazyl (DPPH •), the ability of ginger root extract was determined using a slightly adapted version of the method mentioned by Brand-Viliums *et al* to neutralize. (1995), a function is often used in recent antioxidant studies including ginger extracts. A solution of DPPH was made using a suitable fluid with a strength of 0.1 mm, usually methanol or ethanol (from Sigma-Ladrich, St. Louis, Mo, USA). For testing, a certain amount of extracts of each ginger root were created on a specific strength, quickly interconnected with a fixed amount of DPPH • The solution and reaction remained to react in the dark for 30 minutes to stabilize the solution and reaction. The DPPH radical defect shown by the color change of the solution was measured by checking the absorption at 517 Nm with UV-wise spectrophotometer. A control test where only the DPPH solution (without extract) is served as a baseline reference. We calculated DPPH radical cleansing activity as a percentage using the following formula. To neutralize stable free radical 2,2-diphenyl-picrylhydrazyl (DPPH •), the ability of ginger root extract was determined using a slightly adapted version of the method mentioned by Brand-Viliums *et al* to neutralize. (1995), a function is often used in recent antioxidant studies including ginger extracts (eg al., 2024). A solution of DPPH was made using a suitable fluid with a strength of 0.1 mm, usually methanol or ethanol (from Sigma-Ladrich, St. Louis, Mo, USA). For testing, a certain amount of extracts of each ginger root were created on a specific strength, quickly interconnected with a fixed amount of DPPH • The solution and reaction remained to react in the dark for 30 minutes to stabilize the solution and reaction. The DPPH radical defect shown by the color change of the solution was measured by checking the absorption at 517 Nm with UV-wise spectrophotometer. A control test where only the DPPH solution (without extract) is served as a baseline reference. We calculated DPPH radical cleansing activity as a percentage using the following formula.

DPPH Scavenging Activity (%) = $[A_0 - A_1] \times 100$

Where (A_0) represents the absorbance of the control (DPPH• solution), and (A_1) represents the absorbance of the reaction mixture containing the extract.

3. Assessment of antioxidant capacity using the ABTS radical cation decolorization assay

The total antioxidant capacity of ginger root extracts was evaluated by assessing their ability to hit ABTS • + after a slightly modified protocol based on the first method described by the radical cation, ABTS • + radical cation to 2,2'-Azino bis (3-thylbenzothiazoline-6-sulfonic acid) (ABTS) with 7 mm stem resolution of 2.45 mm Potassium reputation (knee) was allowed. Reacts in the dark at room temperature to secure 12-16 hours.

For the assay, a measured volume of each ginger root extract, at a specific concentration, was rapidly mixed with a defined volume of the diluted ABTS•+ solution and incubated at room temperature for 6 minutes. The reduction in absorbance at 734 nm, indicative of the neutralization of the ABTS•+ radical cation by the antioxidants present in the extracts, was then measured. A control sample containing the ABTS•+ solution without the extract served as the

reference. The ABTS radical scavenging activity, representing the antioxidant capacity, was calculated as a percentage of ABTS•+ radicals scavenged using the following formula:

ABTS Scavenging Activity (%) = $[A_0 - A_1] \times 100$

Where (A_0) represents the initial absorbance of the ABTS•+ solution, and (A_1) represents the absorbance of the reaction mixture after the addition of the extract.

Results

Table 1 presents a comparative analysis of the antioxidant efficacy of *Zingiber officinale* (ginger) root extracts obtained through three distinct extraction methodologies: Clevenger distillation yielded (modified for enhanced volatile recovery), ethyl acetate solvent extraction, and aqueous extraction. For each extract, the total antioxidant capacity (assessed via ABTS assay), the ability to scavenge free radicals (quantified by the DPPH assay), and the reducing power (measured by the FRAP assay) were determined. The results are expressed as the mean value standard deviation.

Table 1: Antioxidant properties of *Zingiber officinale* root extracts obtained by different methods

Reducing Power	DPPH	Antioxidant Capacity	Type of Extraction
90.3385±0.059	90.818±0.017	90.9448±0.076	Clevenger method
88.7217±0.056	85.791±0.011	80.8314±0.050	Ethyl acetate
70.5320±0.048	71.038±0.022	40.3340±0.090	Aqueous extract

The antioxidant potential of *Zingiber officinale* (ginger) root extracts, obtained through Clevenger method extraction (representing our optimized oil extraction), ethyl acetate extraction, and aqueous extraction, was quantitatively evaluated using Reducing power, DPPH radical scavenging activity, and Antioxidant capacity assays (Table 1). The results consistently demonstrated the highest antioxidant potential in the Clevenger method extract (Reducing power: 90.3385±0.059; DPPH: 90.818±0.017; Antioxidant capacity: 90.9448±0.076), followed by the ethyl acetate extract (Reducing power: 88.7217±0.056; DPPH: 85.791±0.011; Antioxidant capacity: 80.8314±0.05), with the aqueous extract exhibiting the lowest activity across all parameters (Reducing power: 70.532±0.048; DPPH: 71.038±0.022; Antioxidant capacity: 40.334±0.09). This clear hierarchy underscores the significant influence of solvent polarity on the extraction efficiency of different antioxidant compounds present in ginger.

The superior antioxidant activity observed in the ginger oil extract obtained via our modified Clevenger method, incorporating specific refinements to temperature and extraction duration, demonstrates the effectiveness of our optimized approach in selectively recovering potent lipophilic antioxidants. The high Reducing power (90.3385±0.059) and DPPH radical scavenging activity (90.818±0.017) achieved with our method significantly surpass those obtained with ethyl acetate (Reducing power: 88.7217±0.056; DPPH: 85.791±0.011) and aqueous extraction (Reducing power: 70.532±0.048; DPPH: 71.038±0.022). The substantial Antioxidant capacity (90.9448±0.076) further confirms the enhanced extraction efficiency of our modified Clevenger method for these lipophilic compounds. This improvement likely stems from the tailored parameters of our method, which appear to minimize degradation and maximize the solubility of key

antioxidant constituents compared to conventional Clevenger extraction.

The intermediate antioxidant activity observed in the ethyl acetate extract (Reducing power: 88.72171 ± 0.056 ; DPPH: 85.791 ± 0.011 ; Antioxidant capacity: 80.8314 ± 0.05) reflects its capacity to solubilize compounds of intermediate polarity, contributing to a less potent overall antioxidant profile compared to our optimized oil extract. This aligns with Ahmad *et al.* (2022), who reported considerable antioxidant activity in ethyl acetate extracts, albeit generally lower than less polar extracts. The consistently lower values in our ethyl acetate extract compared to our Clevenger method extract across all assays highlight the advantage of our specific modifications in targeting lipophilic antioxidants.

The significantly lower antioxidant activity of the aqueous extract (Reducing power: 70.532 ± 0.048 ; DPPH: 71.038 ± 0.022 ; Antioxidant capacity: 40.334 ± 0.09), consistent with the findings of Hossain *et al.* (2023) [29], underscores the limited efficacy of polar solvents in extracting the primary lipophilic antioxidants from ginger root. The stark contrast in Antioxidant capacity between the

aqueous extract (40.334 ± 0.09) and our Clevenger method extract (90.9448 ± 0.076) clearly demonstrates the success of our optimized oil extraction in selectively recovering these valuable compounds. The enhanced performance achieved through our modified Clevenger method, therefore, highlights the critical importance of carefully tuning extraction parameters to maximize the recovery of target bioactive compounds based on their physicochemical properties.

Table 2, chemical composition profile of *Zingiber officinale* root oil by GC-MS

This table presents the percentage composition of various chemical components identified in the oil extracted from *Zingiber officinale* (ginger) roots, analyzed using gas chromatography-mass spectrometry (GC-MS). Compound identification was primarily achieved through comparison with spectral libraries, such as NIST or Wiley, providing a quantitative profile of volatile and semi-volatile constituents that contribute to the characteristic aroma, flavor, and bioactivity of ginger root oil.

Table 2: Chemical composition profile of *Zingiber officinale* root oil

%	Library/ID
19.7868	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, [S-(R*,S*)]-
18.835	Benzene, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
14.1866	Cyclohexene, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R*,S*)]-
6.6632	Beta-Bisabolene
6.5942	Beta-Bisabolene
4.6761	Ambrein
4.2246	Ambroxan or Ambre Oxide
2.4458	trans-Nerolidyl formate
1.97	Sabinene
1.7488	6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl-
1.747	Citral (Geranial (E))
1.7301	(4aR,8aS)-4a-Methyl-1-methylene-7-(propan-2-ylidene)decahydronaphthalene
1.5973	Borneol
1.4513	(1R,4R,4aS,8aR)-4,7-Dimethyl-1-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene
1.418	Phenol, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-, (R)-
1.3233	3-Cyclohexen-1-ol, 1-(1,5-dimethyl-4-hexenyl)-4-methyl-
1.2558	Neral (Citral (Z))
1.0624	Spiro[4.5]dec-6-en-8-one, 1,7-dimethyl-4-(1-methylethyl)-
0.9241	(E)-1-(6,10-Dimethylundeca-5,9-dien-2-yl)-4-methylbenzene
0.8838	4-Isopropyl-6-methyl-1-methylene-1,2,3,4-tetrahydronaphthalene
0.7444	Camphene
0.7386	7-Isopropyl-2,10-dimethyl-1,5-dithia-spiro[5.5]undecane 1-oxide
0.7207	alpha-Terpineol
0.6897	(1R,4R,4aS,8aR)-4,7-Dimethyl-1-(prop-1-en-2-yl)-1,2,3,4,4a,5,6,8a-octahydronaphthalene
0.6744	Bicyclo[3.1.0]hex-2-ene, 4-methylene-1-(1-methylethyl)-
0.6665	Sabinene
0.6368	Beta-Bisabolene

The detailed GC-MS analysis presented in Table 3-2 unveils a complex chemical profile of *Zingiber officinale* root oil, significantly impacting its potential applications in both the flavor and nutraceutical industries. The prominent presence of oxygenated sesquiterpenoids, represented by compounds such as trans-Nerolidyl formate (2.4458%) and 6,10-Dodecadien-1-yn-3-ol, 3,7,11-trimethyl- (1.7488%), alongside the substantial non-oxygenated sesquiterpenoids like the various isomers of Beta-Bisabolene (totaling 13.2574%) and the significant 1,3-Cyclohexadiene derivatives (totaling 52.8084%), suggests a unique aromatic and bioactive signature potentially attributed to our optimized extraction methodology. These groups of constituents are likely key contributors to the characteristic

spicy and warm notes of ginger oil, aligning with traditional uses and potentially offering novel flavor dimensions for culinary applications. Moreover, sesquiterpenoids, both oxygenated and non-oxygenated, are recognized for their diverse biological activities, including anti-inflammatory and antioxidant effects (da Silva *et al.*, 2020) [6]. The enriched levels of these compound classes in our extract suggest enhanced potential for nutraceutical applications.

The significant detection of diterpenoids, specifically Ambrein (4.6761%) and Ambroxan (4.2246%), introduces an intriguing aspect to the chemical composition. While traditionally valued in the fragrance industry for their amber-like and woody notes, their substantial presence in our ginger root oil extract indicates that our extraction

technique efficiently recovers less volatile, higher molecular weight components. This finding could imply a broader spectrum of bioactive compounds being extracted compared to conventional methods, potentially contributing unique flavor nuances or even synergistic effects with other identified constituents, including the aforementioned sesquiterpenoids. Diterpenoids have also been reported to possess various biological activities, further enhancing the potential of our extract.

The identified monoterpenes and oxygenated monoterpenes, such as Sabinene (totaling 2.6365%), Camphene (0.7444%), Geranial (E) (1.747%), Neral (Z) (1.2558%), and alpha-Terpineol (0.7207%), corroborate the expected pungent aroma of ginger. Consistent with the findings of Nascimento *et al.* (2020), these compounds also possess reported antimicrobial and antioxidant activities. The specific ratios of these components achieved through our innovative extraction process may contribute to a more nuanced and desirable flavor profile for various food applications, potentially offering added preservative benefits due to their antimicrobial properties.

When comparing our results with recent literature, the study by Gohil *et al.* (2023) on the volatile profile of ginger essential oil reported lower relative abundances of total sesquiterpenoids and did not identify Ambrein or Ambroxan as significant components. This discrepancy suggests that our modified hydrodistillation technique may be more effective in extracting and preserving a wider range of these compound classes. Similarly, while Sharif *et al.* (2024) focused on the fixed oil of ginger and identified different dominant compounds, the distinct volatile profile obtained in our study, particularly the significant presence of both sesquiterpenoids and diterpenoids like Ambrein and Ambroxan, highlights the selectivity and potential advantages of our hydrodistillation approach in capturing a unique set of bioactive molecules. The alignment of our monoterpene and oxygenated monoterpene findings with the known contributions of these compounds to ginger aroma and bioactivity, coupled with the novel co-occurrence of significant levels of both sesquiterpenoids and diterpenoids, hints at potential synergistic bioactivities or unique flavor profiles not typically observed in conventionally extracted ginger oil. This warrants further investigation into the specific contributions of these diverse compound classes to the overall bioactivity and sensory properties of ginger root oil. In conclusion, our refined extraction of *Zingiber officinale* root oil yielded a rich profile of sesquiterpenoids, notably enriched with Beta-Bisabolene, and uniquely featuring Ambrein and Ambroxan. This suggests our optimized method surpasses conventional techniques in capturing a broader spectrum of bioactive molecules. The resulting oil presents significant potential for functional food development due to its antioxidant and anti-inflammatory properties, and offers novel aromatic possibilities for the flavor industry. The presence of key monoterpenes further contributes to its characteristic aroma and potential antimicrobial benefits. Our findings underscore the importance of tailored extraction strategies for maximizing the recovery of valuable phytochemicals, paving the way for innovative applications in food and health. Further research into the specific bioactivities of this unique extract is warranted.

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