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Research Article

Preparation of Bioactive Zingiber Zerumbet Nanoparticles for Suppressing Cancer Cell

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ABSTRACT

The research was to evaluate the rhizome of *Zingiber zerumbet* grown in India and to evaluate the antibacterial and anti-proliferative potential of the rhizome and its major constituents. It is mainly aimed for its therapeutic and bioimaging applications. Although there are many constituents were identified representing 98.0% of the essential oil composition. Zerumbone (69.86%), α-humulene (6.9%), camphene (5.04%), humulene oxide I (2.45%), humulene oxide II (1.8%), and camphor (1.41%) were the major constituents. The potential of the rhizome essential oil of *Z. zerumbet* and zerumbone was tested against pathogenic bacterial strains. The results showed that both essential oil and zerumbone, possessed significant antagonist activity against *Staphylococcus aureus*-96 (MIC: 51.89 –156.6 µg/mL), *Streptococcus mutans* (MIC: 68–258.0 µg/mL) and *Escherichia coli* (MIC: 94.1–198.0 µg/mL). Zerumbone was found more active compared to the essential oil. Moreover, the antiproliferative potential of *Z. zerumbet* oil and zerumbone was evaluated against various human cancer and normal cell lines (MDAMB-231, A431, WRL-68). Results showed that, both essential oil and zerumbone possessed antiproliferative activity against tested cell lines, where zerumbone was more competent then essential oil.

KeyWords: Anti-Bacterial, Anti Proliferative, Bacterial Strains, Zingiber Zerumbet, Zerumbone.

INTRODUCTION

Recent developments in the field of nanotechnology have demonstrated the need for new development techniques to create novel materials that meet the extremely demanding market sector requirements as well as their potential industrial scalability[1].

The development of nanocomposites containing metallic nanoparticles has gained attention in the last years. This has allowed different researchers to obtain unique geometries with narrow size distributions.

The scientific underpinnings of their therapeutic effects must be established since they can be used to build safe and effective medications. Therefore, it is therefore advised in herbal medicine to investigate the chemical makeup of this plant. In order to determine the chemical makeup of the various plant components of Zingiber zerumbet, including the leaves, cones , roots and rhizomes which are all grown in the foothills of Uttarakhand under ideal agroclimatic conditions, this study was carried out.

With the background of Antibacterial and Anticancerous potential of the essential oil of Z. zerumbet and no much investigations about the activities of Z. zerumbet grown in foothill agroclimatic conditions in northern India, the present study was planned to explore the biological activity of the rhizome essential oil of Z.zerumbet from northern India [2].

The present study was designed to evaluate the following objectives:

(i) To explore the rhizome essential oil composition

(ii) To isolate and characterize the major constituent by chromatographic and spectrometric analysis;

(iii) To evaluate the antiproliferative activity against various organ specific cell lines

(iv) And to evaluate the antimicrobial activity against three pathogenic bacterial strains to have an inclusive view to use the rhizome oil of Z. zerumbet, as alternatives, in microbial and cancer control therapy in humans[3]

EXPERIMENTAL WORKS

Crude MEOH extract

Standard: Zerumbone standard (Sigma Aldrich, 99% W/v) stock solution (1mg/mL) was prepared in MEOH.

Test Sample: Zerumbone content was done in liquid-liquid methanolic fraction and in hexane fraction

HPLC analysis was carried as per the method of as summarized below.

HPLC condition: Instrument: Shimadzhu LC-Prominence 20AT, II. Column: C18 column 250 mm x 4.6 mm, 5u particle ,III. Mobile Phase: Linear,A: HPLC grade ACN (20%), B: HPLC grade Water (80%),IV. Flow Rate: 1ml/min, V.Injection volume: 10ul, VI. Absorbance: 280 nm, Zerumbone was quantified using the peak area after HPLC analysis.

Analysis and Characterization of Essential Oil Constituents

The chemical composition of essential oil was analysed by gas chromatography [4](GC) and gas chromatography-mass spectrometry (GC–MS) techniques.Column: DB-5 capillary column (30 m × 0.25 mm i.d., Film thickness :0.25 µm) fixed inside the oven of NUCON Gas Chromatograph (model 5765).Column Oven temperature: 60 to 230 °C, at the rate of 3 °C min^{-1} , using Hydrogen as carrier gas at constant flow rate of 1.0 mL min^{-1}.

Isolation and characterization of zerumbone

The major constituent of the essential oil was isolated by column chromatography and crystallization process. The purity of the isolated compound was checked using thin layer chromatography (TLC) and gas chromatography (GC) (Purity > 98.0%). The compound was characterized as zerumbone by IR spectral data[5]

Antibacterial activity evaluation of the essential oil

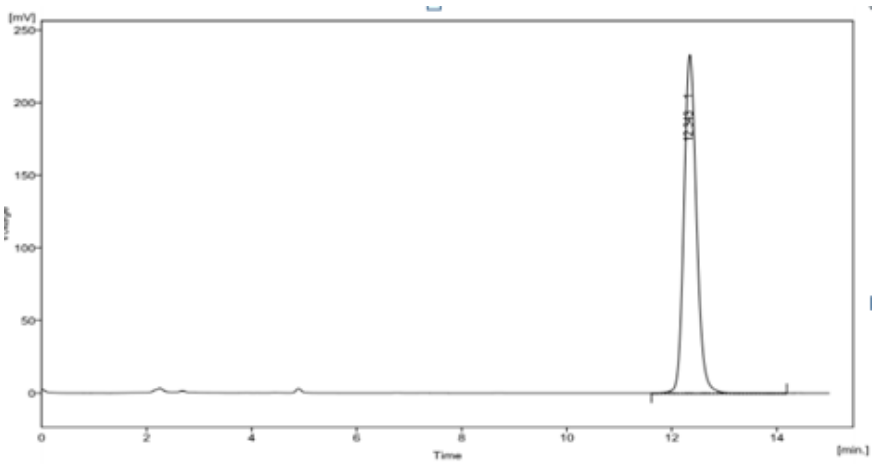
The antibacterial activity of the rhizome essential oil of Z. zerumbet and is major constituents, zerumbone was determined using disc diffusion assay (CLSI, 2006). Inoculum of the three test bacteria viz. Staphylococcus aureus (MTCC 96), Streptococcus mutans (MTCC 890), Escherichia coli (MTCC 723) was prepared equivalent to McFarland Standard 0.5 (1 × 106 cfu/mL) obtained from the Microbial Type Culture Collection Centre (MTCC), Institute of Microbial Technology (IMT) Chandigarh, India

Anti Proliferative activity evaluation of Zerumbone and Essential oil

Antiproliferative evaluation of zerumbone and essential oil. The human cell lines MDA-MB231 (Breast adenocarcinoma), A431 (Skin Carcinoma) and WRL-68 (Hepatic carcinoma) were procured from National Centre for Cell Science, Pune, India. The cells were grown in DMEM. The MTT assay for essential oil and isolated compound was performed according to the method of Mosmann, (1983). The experiment was performed in 96-well plate[6].

Preparation of Nanocomposite Zerumbone

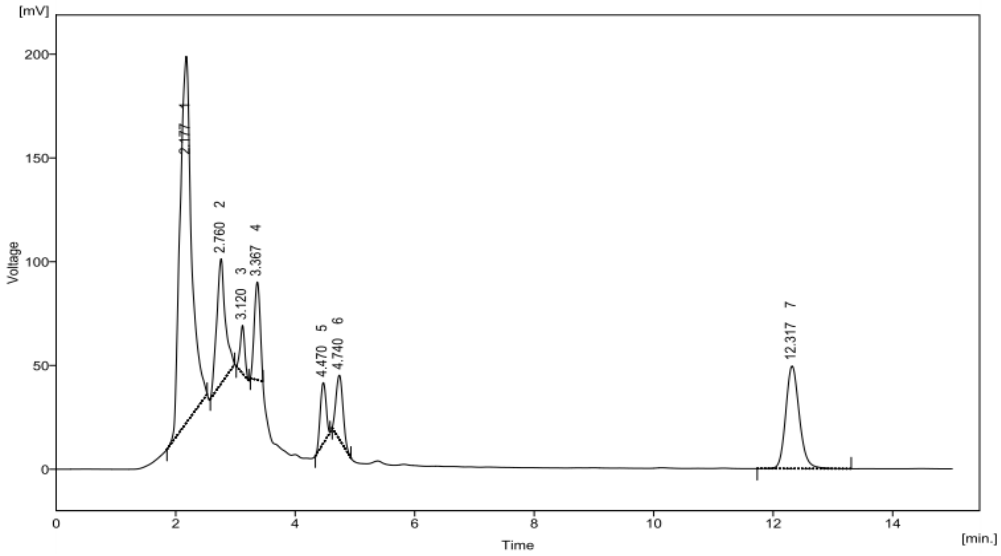
In this research, zerumbone, a physiologically active component that was combined with AuNPs inside of an acrylic polymer by using various thermal treatments[7,8] after production, was the subject of the investigation. With this method, the additive preparation of the object and the creation of the AuNPs is separated into two distinct phases[9], enabling the binding and independent control of these two processes for the development of nanocomposites. By using UV-Vis spectroscopy, these nanocomposites macroscopic optical characteristics were investigated. The different nanocomposites prepared were characterized via scanning electron microscopy (SEM). These nano composites are excellent options for the detection of biomarkers since their development makes it possible to generate transparent substrates with their properties and complicated geometries that can be applied to any surface.



Result Table (Uncal - C:\SPINCHROM\WORK1\DATA\2020\SEPTEMBER\ZERUMBONE 0.25MG.)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	12.343	3865.440	233.053	100.0	100.0
	Total	3865.440	233.053	100.0	100.0

Figure1: HPLC chromatogram for Zerumbone Standard (250µg/ml)



Result Table (Uncal - C:\SPINCHROM\WORK1\DATA\2020\SEPTEMBER\LLFM)

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]
1	2.177	2372.070	176.613	51.1	42.4
2	2.760	601.411	60.111	12.9	14.4
3	3.120	123.304	23.116	2.7	5.5
4	3.367	317.559	47.253	6.8	11.3
5	4.470	196.815	29.274	4.2	7.0
6	4.740	250.213	30.909	5.4	7.4
7	12.317	783.486	49.435	16.9	11.9
	Total	4644.859	416.711	100.0	100.0

Figure 2: HPLC chromatogram for Liquid Liquid fraction by MeoH

Sample	Stock	R.T	Area (mv*S)	ZERUMBONE content ug/mlof extract	Dilution factor	ZERUMBONE content mg/g of extract	Chromatogram Reference
ZERUMBONE	0.25mg/ml	12.46	3865	-	-	-	1
Crude MeoH	10mg/ml	12.47	610	31.57	1	0.032	2
LLMF	10mg/ml	12.31	783	40.52	1	0.041	3
LLHF	10mg/ml	12.3	181	9.37	1	0.009	4

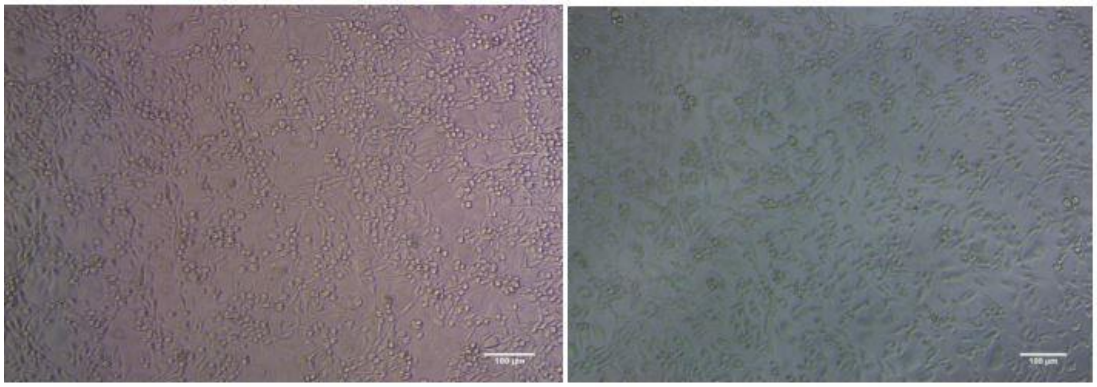
Table 1: **Zerumbone Content of Test Sample**

Bacterial Strains	Essential Oil		Zerumbone		*Standard	
	ZI (MM)	MIC (ug/mL)	ZI (MM)	MIC (ug/mL)	ZI (MM)	MIC (ug/mL
Staphylococcus Aureus (MTCC 96)	9 ± 1	156.6 ± 40.6	10± 2	51.89 ± 10.20	25 ± 1	0.50 ± 0.51
Escherichia coli (MTCC 723)	4 ± 1	198 ± 32	6 ± 1	94.1 ± 10.7	12 ± 1	0.19 ± 0.0
Streptococcus mutans	5 ± 1	258 ± 42	8 ± 2	68 ± 1.6	27 ± 1	0.19 ± 0.16

*ZI: zone of inhibition; MIC: minimum inhibitory concentration. a Standard: Norfloxacin; Data are expressed as mean ± standard deviation

Staphylococcus aureus-96 (MIC: 51.89.–156.6 µg/mL), Streptococcus mutans (MIC: 68.0–198.0 µg/mL), and Escherichia coli (MIC: 94.1–198.0 µg/ mL).

Table 2: Antibacterial Potential of Essential oil of Zingiber Zerumbet and Zerumbone



(A) **(B)**

Figure 3:A-Untreated Cancer Cell, B-Treated Cancer Cell with Zerumbone Nanocomposite

Cell Lines	MDAMB-231(Breast Carcinoma)	A431 (Skin Carcinoma)	WRL-68(Hepatic Carcinoma)
Zerumbone	15.45 ± 0.98	21.75 ± 0.76	30.09 ± 1.1
Essential Oil	34.13 ± 0.91	36.17 ± 1.11	40.78 ± 1.6
Doxorubicin	2.50 ± 0.04	4.6 ± 0.2	2.63 ± 0.03

*The inhibitory concentration (IC50) in µg/mL was calculated

* Organ specific cancer and normal cell lines, MDAMB-231 (Breast Carcinoma); A431 (Skin Carcinoma); WRL-68 (Hepatic carcinoma); IC50 (µg/mL) of antiproliferative activity assay against tested cell lines

Table 3: Antiproliferative Potential of Essential oil of Zingiber Zerumbet and Zerumbone

RESULTS AND DISCUSSION

Zerumbone content was found to be 31µg/mL in crude, 40.52 µg/mL in liquid-liquid methanolic fraction and 9.37 µg/mL in hexane fraction, Table**1.** The rhizome essential oil of Z. zerumbet and zerumbone was tested for antagonist activity against three pathogenic bacteria. The results in terms of zone of inhibition (ZI) and minimum inhibitory concentration (MIC) are presented in **Table 2.** Results showed that both essential oil and zerumbone exhibited varying degree of antibacterial activity against tested strains.

In the present investigation hepatic, breast and skin were treated with different concentration of essential oil and zerumbone (10–50 µg/mL) for 24 h revealed that the proliferation of tested cells were strongly inhibited by the zerumbone, followed by the essential oil in **Table 3.** Zerumbone decreased the proliferation of cancer cells after 24 h incubation, **Figure 3**

CONCLUSION

Specifically, the MTT assay, whose optical density is proportional to the number of viable cells, was used to observe the ability of living cells with active mitochondria to split the tetrazolium ring. It is advantageous to produce natural derived treatments because of zerumbone's documented biological actions, which are diverse. This means that Z. zerumbet, which is grown in India and has a high yield of essential oils and zerumbone, might be used to produce this phyto-molecule, which is widely used in medicinal products.

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