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Antioxidant, antiproliferative and antimicrobial activities study of Zingiber roseum rosc., an endangered medicinal plant

Biotechnology

KEYWORDS : Zingiber roseum, Rhizome, Antibiotic, Antioxidant, Antiproliferative.

Swarnalatha Pakalapati Research scholar, Department of Biotechnology, GITAM Institute of Science, GITAM University, Rushikonda, Visakhapatnam, Andhrapradesh, India 530045.

Ganesh babu Devara Research scholar, Department of Biochemistry, GITAM Institute of Science, GITAM University, Rushikonda, Visakhapatnam, Andhrapradesh, India 530045

Anitha Mamillapalli Associate professor, Department of Biotechnology, GITAM Institute of Science, GITAM University, Rushikonda, Visakhapatnam, Andhrapradesh, India 530045.

ABSTRACT

Medicinal plants are incredible; but many species are endangered due to rapid deforestation and global warming threatens. Zingiber roseum, is an endangered medicinal plant used for skin infections by the traditional healers of Eastern Ghats, India. Chloroform extract of Z. roseum rhiozome is exhibiting a proved antimicrobial activity against four different bacteria, with lowest MIC of 20 µg/ml against Staphylococcus aureus (MTCC 3160). The extract also have efficient radical scavenging activity along with reducing property by DPPH radical scavenging activity, nitric oxide scavenging activity and reducing power assay. The rhizome extract show a moderate antiproliferative activity against PC3 (Prostate cancer), SW 480 (Colon cancer), MDA MB-231 (Breast metastatic) cancer cell lines when IC 50 values compared with standards. The study concludes that the chloroform extract may be an alternative for synthetic therapeutics for treating infectious and degenerative diseases.

Introduction

In the recent past, the research on medicinal plants has been increasing due to the therapeutic potentials of the plants. The use of medicinal plant extracts as therapeutic agents is wide spread since microorganisms and cancers developing resistance to synthetic drugs. The need for new agents against oxidative stress that leads to cancers is growing. Prostate cancer (PCa) is the most common and second most frequent cause of cancer related death in men (Jemal et al., 2006). Recently, the plant derived taxanes [paclitaxel or docetaxel (DTX)] with other agents, such as EMP or predonisone have been used for hormone-resistant prostate cancer (HRPC) (Obasaju C et al., 2001). Worldwide, breast cancer is the most common invasive cancer in women and it comprises 22.9% of invasive cancers in women. Breast cancer causes about 90% of deaths at advanced metastatic stage (Bendre et al., 2003). According to Kang et al., metastasis in human breast carcinoma causes the bone and lung carcinomas (Kang et al.,2003). Colorectal cancer is a carcinoma of parts of the large intestine or in the appendix. Worldwide 1.23 million new cases of colorectal cancer are clinically diagnosed (Jamal et al., 2011).

The ingestion of fresh fruit, vegetables and tea rich in natural antioxidants has been associated with prevention of cancer and cardiovascular diseases (Willcox *et al.*, 2004; Johnson, 2001). Approximately 60 % of the commercially available anti-tumor and anti-infective agents are of natural origin (Cragg *et al.*, 1997).

Abundant plant species are available and there is an urgent need to elevate the therapeutic properties of plants. India is one of the richest reservoirs of biological diversity in the world and is home to a great variety of ethno-medicinally important plant species. However, several medicinal plants in India are at risk of extinction, *Ziniber roseum* Rosc. (*Zingiberaceae*) is one of the important endangered medicinal herbs scattered widely in Himalayan region and Eastern Ghats (Babu, 1977). The rhizomes of *Z.roseum* have been used by tribal communities in Central India for treatment of fever and rheumatism (J.K. Maheshwari *et al.,* 1986) (N.C. Babu *et al.,* 2010). Antifertility and myorelaxant activity of stem extract has been reported (A.O. Prakash *et al.,* 1993) (C. Pattanaik *et al.,* 2009).

In the present study, anticancer, antioxidant and antimicrobial activities of *Z.roseum* was under taken. Secondary metabolites which show more than one therapeutic properties are more advantageous like essential oil of *Mesua ferrea* leaves, it is having the antimicrobial and anti oxidant activities along with anticancer properties (Sukanya and Samart, 2012). Multi-therapeutic properties of a single drug will be beneficial to overcome the secondary infections in cancer patients and also an alternative for treatment of MDR bacterial strains. This is the primary report stated on anti bacterial, anti oxidant and anti cancer properties of *Zingiber roseum* crude rhizome extract.

Materials and Methods

Gram-positive bacterial strains *Staphylococcus aureus* (MTCC 3160), *Streptococcus* pyogenes (MTCC 442). and Gram-negative bacterial strains *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 424) are obtained from the (MTCC) Microbial Type Culture Collection, Chandigarh for antibiotic assays.

Human cancer cell lines PC3 (Prostate Cancer), SW480 (Clon Adeno Carcinoma), MDA-MB 231 (Breast Cancer) are procured from National Centre for Cell Science, Pune.

Preparation of plant extract

Plant material was collected from Rampa chodavaram, Eastern ghats. Specimen was deposited in Andhra University Herbarium and no was A.U. (B.D.H.) 20376. The rhizomes are air dried, chopped and crushed and mixed with chloroform at room temperature. After 48 hrs of soaking, the extract was filtered and evaporated to dry using rotor evaporator. The residue was weighed and suspended in 5% DMSO in PBS to get 1 mg/ml concentration.

Antibacterial activity

Antimicrobial susceptibility testing

For the antibacterial tests, organisms are grown overnight in LB broth followed by incubation at 37ºC. (Firas A. AL-BAYATI, 2008). The antimicrobial activity of chloroform extract of rhizome was determined by using a modified agar well diffusion method (Chanpen Chanchao, 2009).

A loop full of overnight grown microorganisms was uniformly distributed on the surface of the agar plate by spreading with a sterile swab. Wells of approximately 4mm in diameter and 2.5mm depth are made on the surface of the solid medium using a sterile borer (Apak Lino, 2006) and each well loaded different concentrations of the plant extracts (10, 20,30,40 µg/ml) control well loaded with 20 µl of chloroform. The concentration of the extracts employed are 10, 20, 30, 40 µg/ml. Blank well was filled with 20 µl of chloroform and Ampicillin 40 µg/ml was used as a positive control (Jhon J Rojas, 2006). The test was carried out in triplicates. The plates are incubated at 37°C for 24 hrs and the Zone of inhibition was measured.

Determination of minimal inhibitory concentration (MIC)
A single colony of bacteria was transferred for overnight growth culture into a 100 ml of mueller hinton broth and incubated overnight at 35 °C. When the OD of the culture was 0.5-0.8, cells are spun down at 4000 rpm for 5 min taking appropriate precautions. The pellet was finally suspended in sterile normal saline.

The resazurin solution was prepared by dissolving a 270 mg tablet in 40 ml of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution.

A homogeneous solution was prepared by dissolving resazurin tablet (270 mg tablet in 40 mL water). A sterile 96 well plate was labeled and bacterial cells of known number are added. To the wells, serial dilutions of the test material was added with a multichannel pipette. To each well 10 µL of resazurin indicator solution was added. Final volume of each well was made to 150 µL with Muller Hinton broth. Each plate had an antibiotic as positive control (usually ampicillin in serial dilution). Two negative controls and one positive control are kept for each plate. Ampicillin is used as positive control and one well without bacterial cells and the other without the test compound are kept as negative controls. Plates are incubated at 37 °C for 18–24 h. Colour changes was recorded using ELISA reader. All the dilutions, positive and negative controls are done in triplicates. Any colour changes from purple (oxidized) to pink (reduced) or colour less are recorded as positive. The lowest concentration of each drug that prevented this colour change was measured as MIC (F. Ang, 2010). The average of three values was calculated and that was the MIC for the test material and bacterial strain.

Antioxidant activity
Determination of DPPH radical scavenging activity
The free radical scavenging activity was measured in terms of hydrogen donating or radical-scavenging ability using the stable radical (Deepika Gupta *et al.,* 2011). To the 3ml of extract at different concentrations (50-250 µg/ml), 0.1 mM DPPH solution in ethanol was added. Thirty minutes later, the absorbance was measured at 517 nm. Butylated Hydroxy Toluene(bht) was used as a standard. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH Scavenged (\%)} = (A_c - A_t / A_c) \times 100$$

A_c = control reaction absorbance

A_t = extracts or the standard chemical absorbance The IC50 value was defined as the concentration of the extract in µg/ml of that inhibits the formation of radicals by 50%.

Reducing power assay
The reducing power of methanolic extracts was determined according to the method of (Isabel C.F.R. Ferreira, 2007). Different concentrations of plant extract (25– 125 µg/ml) in methanol are mixed with 2.5ml of phosphate buffer and 1% potassium ferrocyanide. Trichloroacetic acid (10%) was added after twenty minutes of incubation at 50°C and centrifuged at 3000 rpm for 10 min. The upper layer and distilled water are mixed in equal amounts and 0.1% of FeCl3 was added. The absorbance was measured at 700 nm. Absorbance values increased with increase in the reducing power of the extract.

Nitric Oxide scavenging activity
Nitric Oxide radical scavenging activity was estimated by the use of Griess Illosvoy reaction (Garrat, 1964). Griess reaction measures the Nitric oxide generated from sodium nitroprusside. Nitric oxide scavenger reduces the production of nitric oxide. Scavenger of nitric oxide competes with oxygen leading to reduced production of nitric oxide. Sodium nitroprusside (5 mM) in phosphate–buffered saline (PBS) was mixed with 3.0 ml of different concentrations (5-25 µg /ml) of the drugs dissolved in the suitable solvent systems and incubated at 25^{0}C for 150 min. The samples from the above are reacted with Griess.The absorbance of the formed chromophore was measured at 550 nm.

Anticancer activity
The anticancer activity of *Zingiber roseum* rhizome crude extract was checked on three types of cell lines. Those are PC3 (Prostate cancer), SW 480 (Colon cancer), MDA MB (Breast metastatic) selected for the estimation of cytotoxicity of test compound. PC-3 (PC3) is a human prostate cancer cell line, the cells are useful in investigating the biochemical changes in advanced prostatic cancer cells and in assessing their response to chemotherapeutic agents (Kaighn M.E.*et al.,* 20127). SW 480 cells are epithelial like in morphology and used as an in vitro model for colorectal cancer to study drug efficacy and pharmacodynamics. MDA-MB-231, cells derived from a patient with disseminated breast cancer, they are able to induce metastases to bone and to the adrenal medulla in mice modals (Kang *et al.,* 2003).

Cell culture
Anticancer activity was done by Cell cytotoxicity assay (MTT assay). *Zingiber roseum* rhizome crude extract was checked on four types of cancer cell lines obtained from National Center for Cell Science, Pune, india. those are PC3 (Prostate Cancer) Cell line, SW480 (Colon Adeno Carcinoma) Cell line, MDA-MB231 (Metastatic Breast Cancer) Cell line, MCF-7 (Breast Cancer) Cell line. All cells are grown in Minimal essential medium (MEM, GIBCO) supplemented with 4.5 g/L glucose, 2 mM L-glutamine and 5% fetal bovine serum (FBS) (growth medium) at 37°C in 5% CO_2 incubator.

MTT Assay
Cells gown in T-25 flask are trypsinized and 5 ml of complete media was added. The cells are harvested, counted and diluted to 75,000 cells per ml using complete media. 100 µl of the counted cells are added into each well and incubated overnight. On day-2, cells are treated with different drug concentrations like 12.5, 25, 50, 100, 200 µg/ml. Final volume should be 100 µl per well. On day-3, 20 µl MTT solution (5 mg/ml) was added aseptically to each well. One well with MTT but no cells was kept as negative control. After incubation media was removed carefully, without disturbing the cells and 150µl acid propanol was added to extract the product. Plates are covered with foil and agitated cells on orbital shaker for 15 min. The absorbance is measured at 580nm. In control wells, only suspended cells are added without drug.

Statistical Analysis
The mean values and significance between groups are calculated with the help of Graph pad prism. All results are Mean±SEM.

RESULTS
Antimicrobial activity
The zone of inhibition against *Escherichia coli* at 40µg/ml of extract was 6.6±0. Zone of inhibition against Pseudomonas was 6.73±0.14 mm. ZOI of crude extract against *Staphylococcus aureus was* 7.3±0.19mm at 40µg/ml. The crude extract zone of inhibition against *Streptococcus pyogenus* at 40µg/ml was 7.23±0.11mm.

The negative control DMSO did not show any zone of inhibition, whereas the positive control, ampicillin showed the zone of inhibition 9.8±0.11 mm against *Escherichia coli,* 8.6±0.15 mm against *pseudomonas aeruginosa,* 8.4±0.15 mm against *Staphylococcus aureus*, 9.4±0.11 mm against *Streptococcus pyogenes* at 40µg/ml. **(Fig 1).**

The minimum inhibitory concentration (MIC) assay was assayed using resazurin. The MIC was defined as the lowest concentration of each drug that has no bacterial growth (Ang et al., 2010). Resazurin turns from dark blue to pale pink colour when bacterial growth occurs. The change in colour is measured at 580nm using a colorimeter. High absorbance values correlate with less bacterial growth.

The minimum inhibitory concentration of *Zingiber roseum* crude extract against *E.coli* was 40µg/ml. The minimum in-

hibitory concentration (MIC) of *Zingiber roseum* crude extract against *Pseudomonas aeruginosa* was 40µg/ml and the MIC of *Zingiber roseum* against *Streptococcus pyogenes* was 30 µg/ml. *Zingiber roseum* crude extract showed the lowest MIC at 20 µg/ml concentration against *Staphylococcus aureus* **(Fig 2) (Table 1)**.

Antioxidant activity

Rhizome extract of *Zingiber roseum* showed 52% DPPH raical scavenging activity at 200 µg/ml concentration. Whatever concentration the *Zingiber roseum* crude extract showed 52% scavenging, at the similar concentration BHT percent inhibition was 65% **(Fig 3A) (Table 2)**. The activity of crude extract was comparable to BHT activity. BHT is a food additive used for preservation which is responsible for child hyperactivity (Feingold BF, 1982) and it could be a cause of cancer risk (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 1986).

The Reducing power ability of crude extract was increases with increase in concentrations. Ascorbic acid was taken as standard at similar five concentrations **(Fig 3B) (Table 3)**. The percent reduction of nitric oxide radicals by crude extract at 125 µg/ml concentration was 38.3%, at the same concentration the percent reduction of nitric oxide radicals by ascorbic acid was 45.8% **(Fig 3C) (Table 4).**

Antiproliferative activity

IC 50 value of Z. roseum crude extract against PC3 cell line was 222.224 µg/ml **(Fig 4A) (Table 5)**, and against SW-480 cell line was 583.2689µg/ml **(Fig 4B) (Table 6)**. The IC 50 value of crude extract against MDA-MB231 cell line was 426.378µg/ml **(Fig 4C) (Table 7)**. Crude extract of rhizome showed moderate anti cancer activity against prostate, colorectal, and breast metastatic cell lines when IC 50 values are compared with Standards like paclitaxel showed IC 50 against PC3 was 67.2782 µg/ml, 5-FU IC50 showed IC 50 against SW-480 was 110.618 µg/ml and Tamoxifen showed IC 50 against MDA-MB231 was 426.378 µg/ml.

Discussion

Plant secondary metabolites are more effective against MDR strains when compared to the synthetic preparations. Terpenes are the group of compounds derived from different plant sources having numerous therapeutic properties. The effect of some terpenes on microorganisms has been studying from 1980 onwards (Andrews *et al*., 1980) and proved these compounds shown antimicrobial activities (Islam *et al*.,2003) for example cinnamon oil which having terpenes shown broad-spectrum activity against *Pseudomonas aeruginosa (*Prabuseenivasan *et al*., 2006). The stem bark extract of the cape ash (*Ekebergia capensis)* a native tree of Kenya, contains numerous compounds among those 10 are terpenes which showed antimicrobial activity in vitro (Murata *et al*., 2008). The composition of terpenes differ from one species to another for example *Neolitsea foliosa* plant oil contains only sesquiterpenes like caryophyllene and lack of monoterpenes which also showed some antibacterial properties (John *et al*., 2007). According to the studies all types of terpenes may be act as bactericidal or bacteriostatic agents. Murata found the antimalarial activity of terpenes against *Plasmodium falciparum* (Murata T *et al*., 2008). The antifungal activity of terpenes was also reported against pathogenic yeast (*Candida albicans*) (Morales *et al*., 2003).

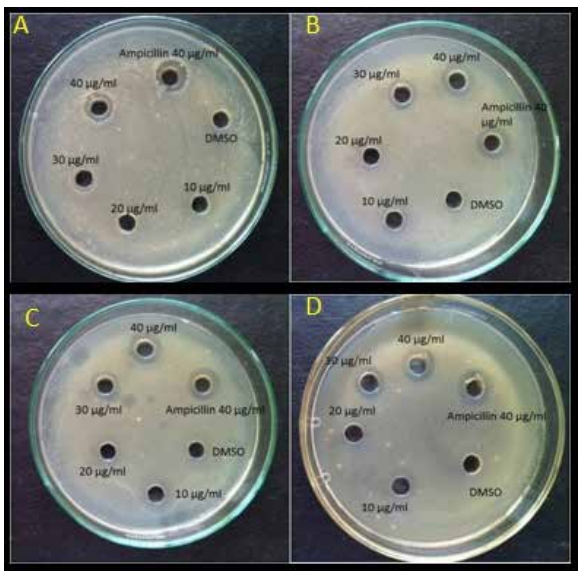
A recent study showed that the plant derived terpenes acts against Methicillin resistant *S. aureus* (MRSA) which is a slime producer and it is able to resist β-*lactamic* antibiotics and where is the conventional chemotherapics are ineffective (Gallucci N *et al.,* 2010). Rhizome extract of *Zingiber roseum* mainly contains terpenoids active against gram negative and gram positive bacteria. The extract was effective when compared with antibiotic activity of other *zingiberaceae* members like Zingiber zerumbet which showed effective zones of inhibition between 128-256 µg/ml against two gram positive and four gram negative bacteria (Golam Kader 2011). *Z. roseum* crude extract showed the lowest MIC at 20 µg/ml concentration against Staphylococcus than remaining tested organisms, which shows *Zingiber roseum* crude rhizome extract was very effective when compared to ginger by the effective zone of inhibition against Pseudomonas aeruginosa at the concentration of 20mg/ml which contains terpenoids (Chen IN, 2008).

Antioxidant effect of natural compounds correlates with the presence of large quantities of phenolics but not always, some authors have reported good linear correlation between the two parameters (Zheng & Wang, 2001; Shan *et al.*, 2005; Djeridane *et al.*, 2006; Katalinic *et al.*, 2006). More recently the terpeniods also found to be beneficial as strong antioxidants (Vinson *et al., 2002*; Wang *et al.,*1997) Terpenes proved as good antioxidants relevant to oxidative stress conditions in different diseases including liver, renal, neurodegenerative and cardiovascular diseases, cancer, diabetes as well as in ageing processes (Gonzalez and Gomez 2012). *Zingiberaceae* family plants are richest sources of natural antioxidants, by this group of plants having terpenoids, phenols and flavonoids.

A study was conducted by Chen IN *et al*, on 11 different species of *Zingiberaceae* family in Taiwan, revealed the antioxidant properties. The activities are best observed in *Vanoverberghia* and *Hedychium*, both species showed 89% antioxidant activity, and DPPH scavenging activity followed similar trends. *Zingiber oligophyllum*, considered as a traditional medicinal plant used in Taiwan exhibited low DPPH scavenging activity and reducing power (Chen IN *et al*,. 2008). Rhizome extract of *Zingiber roseum* showed 52% DPPH radical scavenging activity at 200 µg/ml concentration, this concentration was very effective when compared with *Zingiber montanum* 50% DPPH scavenging activity at 1.0599 mg/ml concentration (Saowaluck 2009) and the rhizome extract showed significant reducing power ability and reduction of nitric oxide radicals when compared with ascorbic acid as standard because. According to this features it may be a good antioxidant. Antioxidants may slow or possibly prevent the development of cancer. *Zingiberaceae* family consists of number of precious medicinal plants like curcumin, zinger etc. and many species used as anticancer therapeutics and proved in vitro and in vivo. Chandra Kirana *et al* (2007) tested anticancer activity of rhizome extracts on 11 *Zingiberaceae* family members. Among those 11 ginger species, ethanol extracts of eight species (*Asmommum cardamomum*, *C. longa, C. mangga, C. xanthorrhiza*, *Boesenbergia pandurata, Zingiber aromaticum, Z. officinale, Z. cassumunar*) showed a strong inhibitory effect on the growth of MCF7 cancer cells with the IC50 concentrations between 10-100 µg/ml. C. longa was proved as a anticancer agent by in vitro, in vivo tests and is currently undergoing clinical trial against colon cancer (Greenwald, et aI., 2001; Sharma et aI., 2001). IC 50 value of Z. roseum crude extract against PC3 cell line was 222.224 µg/ml, and against SW-480 cell line was 583.2689µg/ml. The IC 50 value of crude extract against MDA-MB231 cell line was 426.378 µg/ml **(Fig 4)**.

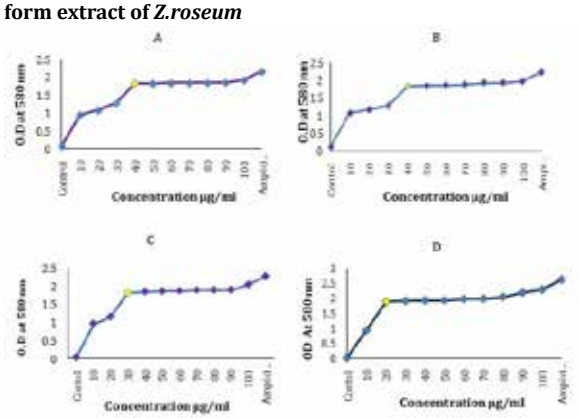
Crude extract of rhizome showed moderate anti proliferative activity against prostate, colorectal, and breast metastatic cell lines when compared with Standards like paclitaxel , 5-FU and tamoxifen which are used in cancer chemotherapy. Chemotherapy is used alone or in combination with other treatments such as radiation or surgery for the treatment of some types of cancers. Chemotherapy drugs reach all parts of the body, not just the cancer cells. Because of this, there may be many side effects during treatment, example **Paclitaxel (Taxol) causes** female infertility (Ozcelik Bulent *et al.,* 2010). **Fluorouracil (5-FU)** cause acute central nervous system damage (Han R *et al.,* 2008). **Tamoxifen is responsible for endometrial cancer** (Gallo MA and Kaufman D, 1997). When the acivty of *Zingiber roseum* hizome extract was moderate this may be an alternative for escaping of these side effects because several studies have proved that plant secondary metabolites an excellent activity against pathogens, cancers and some metabolic disorders. *Zingiber roseum* is also shown such type of activity against oxidative stress, cancer and pathogen. *Zingiber roseum* plant secondary metabolites may full fill the need of new therapeutics at some extent.

Fig 1:- Antibacterial activity of crude chloroform extract of *Z.roseum*.



A:-Zone of inhibition at different concentrations against *Escherichia coli.* B:-Zone of inhibition against *Pseudomonas aeruginosa.* C:- Zone of inhibition at different concentrations against *Staphylococcus aureus.* D:- Zone of inhibition against *Streptococcus pyogenes.*

Fig 2:-Minimum inhibitoy concentration of crude chloroform extract of *Z.roseum*



A:- MIC against *E.coli*. B:-MIC against *P.aeruginosa*. C:-MIC against *S.pyogenes.* D:- MIC against *S.aereus.* All experiments are triplicates (n=3): SE ±, P< 0.05.

Table 1-Minimum Inhibitory Concentrations of *Zingiber roseum*

Concentrations in ug/ml	OD at 580 nm			
	E.coli	S.pyogenes	P.aeugenosa	S.aureus
Control	0.077±0.010	0.050±0.015	0.11±0.011	0.046±0.014
10	0.950±0.029	0.954±0.028	1.073±0.020	0.933±0.013
20	1.083±0.022	1.130±0.014	1.169±0.018	1.842±0.023
30	1.281±0.026	1.825±0.012	1.278±0.010	1.934±0.023
40	1.825±0.011	1.838±0.013	1.835±0.013	1.954±0.016
50	1.827±0.011	1.839±0.010	1.842±0.011	1.948±0.022
60	1.835±0.013	1.848±0.012	1.859±0.010	1.970±0.011
70	1.844±0.013	1.865±0.023	1.876±0.014	2.035±0.038
80	1.848±0.010	1.874±0.017	1.929±0.012	2.060±0.015
90	1.856±0.013	1.914±0.013	1.931±0.014	2.159±0.029
100	1.919±0.011	2.032±0.010	1.965±0.016	2.368±0.045
`Ampicillin 50	2.161±0.026	2.276±0.043	2.227±0.011	2.595±0.047

Minimum inhibitory concentration of *Zingiber roseum* crude extracts. The test compound was effective against gram positive bacteria with very low MICs, than gram negative bacteria. Results are mean of 3 replicas. All experiments are triplicates (n=3): Mean ±SEM, P< 0.05 when compared between gram positive and gam negative. P<0.01 when compared between two gram positive strains and compared between two gram negative strains.

Fig 3A:-DPPH radical scavenging activity of *Z.roseum* crude extract

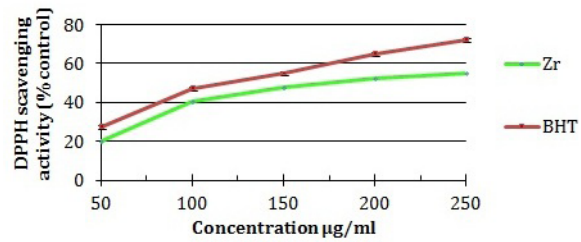


Fig 3B:-Reducing power ability of *Z.roseum* crude extract

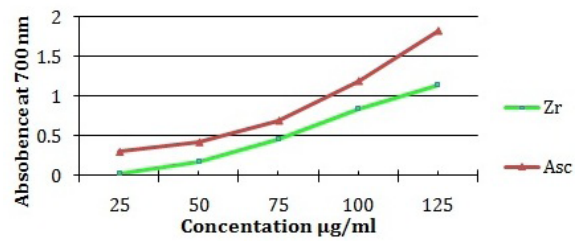
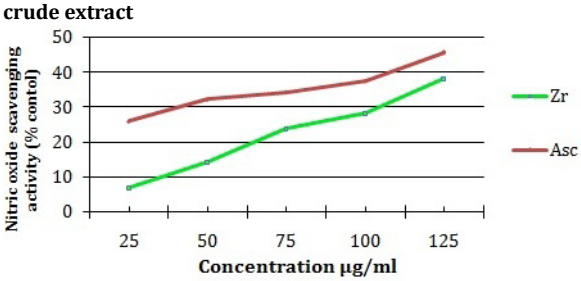


Fig 3C:-Nitric oxide radical scavenging activity of *Z.roseum* crude extract



The O.D values are average of 3 replicas. SE±, P<0.05 when compared between test and standard.

Table 2- *Z.roseum* DPPH radical scavenging activity

conc. In µg/ml	Zingiber roseum		Butylhydroxytoluene (BHT)	
	Absorbance at 517nm	% of Activity	Absorbance at 517nm	% of Activity
50	0.417±0.010	20.05	0.380±0.014	27.5
100	0.283±0.011	40.35	0.254±0.012	47.5
150	0.265±0.014	47.55	0.196±0.012	55
200	0.246±0.012	52.22	0.161±0.014	65
250	0.204±0.011	54.88	0.135±0.010	72.5

All experiments are triplicates (n=3): Mean ±SEM, P< 0.05 when test group compared with standard group

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Table 3- *Z.roseum* Reducing power activity

Conc. In ug/ml	Absorbance at 700nm	
	Zingiber roseum	Ascorbic acid
25	0.026±0.011	0.322±0.015
50	0.174±0.011	0.427±0.014
75	0.472±0.010	0.689±0.011
100	0.846±0.013	1.193±0.005
125	1.147±0.005	1.842±0.011

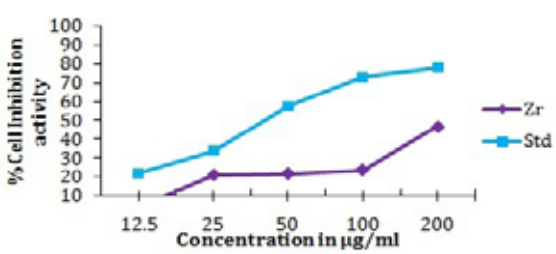
All experiments are triplicates (n=3): Mean ±SEM, P< 0.05 when test group compared with standard group

Table 4- *Z.roseum* Nitric oxide scavenging activity.

Conc. In ug/ml	Absorbance at 550nm	% of Activity Z.roseum	Absorbance at 550nm	% of Activity Ascorbic acid
25	0.158±0.010	6.92	0.128±0.009	26.04
50	0.147±0.010	14.45	0.122±0.010	32.29
75	0.135±0.005	23.9	0.119±0.007	34.37
100	0.122±0.010	28.3	0.116±0.009	37.5
125	0.114±0.007	38.3	0.107±0.009	45.8

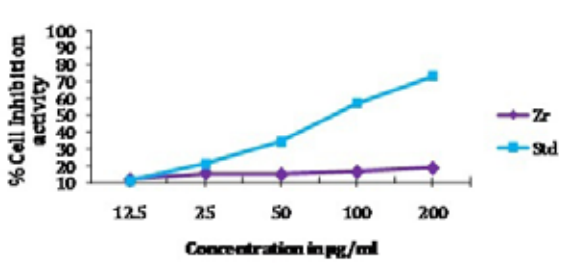
All experiments are triplicates (n=3): Mean ±SEM, P< 0.05 when test group compared with standard group.

Fig 4A:-Cell inhibition of *Z.roseum* extract against PC3 cell line



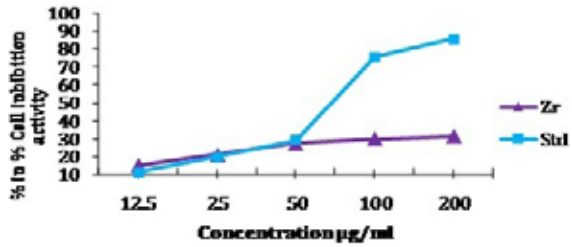
The growth inhibition of *Z.roseum* crude extract against PC3 was compared with paclitaxel

Fig 4B:-Cell inhibition of *Z.roseum* extract against SW-480 cell line



The growth inhibition of *Z.roseum* crude extract against SW-480 compared with standard 5-flouro uracil.

Fig 4C:-Cell inhibition of crude extract against MDA-MB 231 cell line



The growth inhibition of ***Z.roseum*** crude extract against MDA-MB 231 compared with tamoxifen.ss

Table 5- Antiproliferative activity of *Z.roseum* against PC3 cell line.

Conc.ug/ml	OD of Paclitaxel at 580 nm	% Cell Survival	% Cell Inhibition	OD of crude at 492 nm	% Cell Survival	% Cell Inhibition
12.5	0.566±0.015	78.0	22.0	0.850±0.013	95.69	4.3
25	0.446±0.016	66.3	33.7	0.629±0.010	79.13	20.86
50	0.320±0.017	42.4	57.6	0.569±0.024	78.25	21.74
100	0.199±0.011	27.2	72.8	0.438±0.019	76.39	23.6
200	0.180±0.014	21.9	78.1	0.341±0.015	53.37	46.62

The growth inhibition of test compound against PC-3 at different concentrations. All experiments are triplicates (n=3): Mean ±SEM, P> 0.05 when test group compared with standard group.

Table 6- Antiproliferative activity of *Z.roseum* against SW480 cell line.

Con.c ug/ml	OD of 5Flouro Uraclat 580nm	% Cell Survival	% Cell Inhibition	OD of crude at492nm	% Cell Survival	% Cell Inhibition
12.5	0.677±0.014	88.84	11.16	0.707±0.012	92.92	11.75
25	0.524±0.012	78.91	21.09	0.663±0.012	85.09	15.4
50	0.441±0.027	65.69	34.31	0.630±0.008	84.94	15.05
100	0.307±0.019	43.02	56.98	0.621±0.011	83.66	16.33
200	0.238±0.023	26.78	73.22	0.601±0.010	81.01	18.98

The growth inhibition of test compound against SW-40 at different concentrations. All experiments are triplicates (n=3): Mean ±SEM, P> 0.05 when test group compared with standard group.

Table 7- Antiproliferative activity of *Z.roseum* against MDA MB-231 cell line.

Conc. of Extract (ug/ml)	OD At 580nm. Tamoxifen	% Cell Survival	% Cell Inhibition	OD at 492 nm.Crude	% Cell Survival	% Cell Inhibition
12.5	0.664±0.021	88.4	11.16	0.643±0.015	84.87	15.12
25	0.627±0.004	80.1	19.9	0.585±0.011	78.91	21.08
50	0.533±0.017	70.5	29.5	0.458±0.024	72.59	27.4
100	0.240±0.018	24.3	75.7	0.416±0.011	70.11	29.88
200	0.130±0.013	14.4	85.6	0.370±0.009	68.65	31.34

The growth inhibition of test compound against MDA MB-431 at different concentrations. All experiments are triplicates (n=3): Mean ±SEM, P> 0.05 when test group compared with standard group.

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