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Evaluation of *In-Vitro* Immunomodulatory Activity of Ethanolic and Aqueous Extracts of *Zingiber capitatum* Roxb, Dried Rhizomes

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ABSTRACT
The immune system has a fundamental role in protecting the body against pathogenic microbial agents. The function and efficiency of the immune system might be influenced by many exogenous factors such as food, pharmaceuticals, physical and psychological stress, hormones, etc., resulting in either immunostimulation or immunosuppression. Global reliance on an alternative system of medicine for chronic and acute ailments resulted in an intense area of research and discovery of several herbs with the potential to cure diseases. Among them, ample number of herbs have been exploited for modulation of the immune system from medicinal plants. Zingiber capitatum Roxb. is the potential source of natural antioxidant and antimicrobial agents. Present study was aimed to evaluate in-vitro immunomodulatory activity of ethanolic and aqueous extract of Zingiber capitatum Roxb. After phytochemical evaluation; in-vitro immunomodulatory activity was studied by evaluating the release of following immune mediators from murine peritoneal macrophages such as superoxide (NBT reduction), nitric oxide (NO), lysosomal, and myeloperoxidase enzymes. Results showed that, effects of different concentrations of the extract on the superoxide generation in terms of the reduction of NBT dye and nitrite release in the culture supernatant. The extract showed a maximum increase in enzyme activity. Study concluded that ethanol aqueous extract of Zingiber capitatum Roxb. rhizome extract stimulates non-specific immune responses by stimulating macrophages. In-vivo studies also need to be done to correlate with the in-vitro result.
Keywords: *Zingiber capitatum Roxb., in-vitro immunomodulatory activity, nitrite assay, Cellular lysosomal enzyme activity, myeloperoxidase activity, etc.*

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INTRODUCTION

Zingiber capitatum is a herbaceous, perennial plant that spreads by rhizomes and possibly also by seed. It is native to India and is probably also native to Bangladesh and Nepal. *Zingiber capitatum* Roxb is reported different parts of India like Sikkim, Bihar and Bengal and Karnataka. It has medicinal properties and is likely to have been introduced to countries outside its native range for horticultural use. [1, 2] *Zingiber capitatum* is the potential source of natural antioxidant and antimicrobial agents. It shows the activity against both gram positive and gram-negative bacteria indicate the existence of wide spectrum antibiotic compound. *Zingiber capitatum* also have many healing properties like against asthma and cold etc. [2, 4] Preliminary phytochemical analysis revealed the presence of alkaloid, cardiac glycoside, tannin, saponin and carbohydrates.[5] Currently, worldwide, there is an increase in diseases especially infectious diseases that requires efficient body defense mechanisms to control them through the process of immunomodulation.[6] The major causes of immunodeficiency include stress, infectious diseases such as acute respiratory tract infections, diarrheal diseases, yellow fever, hepatitis A and E, tuberculosis, and HIV/AIDS. [7] Various allopathic drugs or medicines are used to modulate the immune system. However, these drugs are very expensive for poor people, they are not easily accessible, and in most cases, they are associated with adverse drug reactions. As a result, the majority of people especially in the rural areas of

the developing world turn to the use of alternative herbal medicines from medicinal plants such as *Zingiber capitatum* Roxb. that are widely accepted, accessible, cheaper, and assumed to have fewer side effects.[7, 8] Modulation of immune responses to alleviate disease has been of interest for many years and is a concept of Rasayana in Ayurveda. Medicinal plants have been used in various traditional systems and have immune potential against various diseases.[9] Hence the present study aimed to evaluate *in-vitro* immunomodulatory activity of ethanolic and aqueous extract of *Zingiber capitatum* Roxb.

MATERIAL AND METHODS

Plant source:

Rhizome of *Zingiber capitatum* Roxb. were purchased from the local Karjat nursery in July 2019 and were authenticated by Dr. (Mrs.) Bindu Gopalkrishnan, Asst. Professor, Department of Botany at Mithibai College of Arts, Chauhan Institute of Science & AmruthbenJivanlal College of Commerce and Economics, Vile Parle (W), Mumbai- 400056 and the voucher specimen (No.MIT0157) were submitted to Pharmacology department, Oriental College of Pharmacy, Sanpada, Navi Mumbai- 400705 for future reference.

Chemicals:

Sulfanilamide and naphthyl ethylenediamine were obtained from Sisco Research Lab Ltd., Mumbai, India. RPMI (Roswell Park Memorial Institute)-1640 was obtained from Himedia Laboratories, Mumbai, India. Ethanol AR, phosphoric acid, and Dimethyl Sulfoxide (DMSO) were obtained from S.D. Fine Chemicals, Mumbai, India. All other chemicals used were of analytical grade.

Methods:

Preparation of Extract:

The rhizomes was cut down into small pieces, shade dried and powdered to get moderately coarse powder, which is sieved under mesh. The powder was then subjected to successive extraction with petroleum ether, water and hydro-alcohol at (60°-80°C), by hot continuous percolation using soxhlet apparatus having 3 cycles each for 24 hours to give petroleum ether extract of rhizome of *Zingiber capitatum* (ZEE), similarly successive extraction with water to give aqueous extract of rhizome of *Zingiber capitatum* (ZAE). The extract were filtered and evaporated to dryness with a dryer. [10, 11]

Phytochemical Evaluation of Extracts:

Phytochemicals are the chemicals that naturally occur in plants. Phytochemical evaluation plays a significant role in the standardization of the crude extracts and was carried out for detecting the presence of various phytoconstituents in the plants under investigation. Various extracts of powdered plant materials were subjected to preliminary phytochemical evaluation using qualitative chemical tests for detecting the presence of the phytoconstituents like alkaloids, glycosides, tannins, phenolic compounds, phytosterols, carbohydrates, proteins, amino acids, etc. [12-14]

In-vitro Immunomodulatory Activity of *Zingiber capitatum* Roxb. Rhizome extract

In-vitro immunomodulatory activity studies for the test extracts were carried out by, screening for release of various immune mediators from isolated murine peritoneal macrophages.

Isolation of peritoneal macrophages and culture conditions

To collect macrophages, 2-ml syringe was filled with 2.0 ml 3% fluid thioglycollate medium (Himedia). Peritoneal cells were washed twice with PBS by discarding supernatant and resuspending the cell pellet by gently pipetting in and out the contents. Cell pellet resuspends in RPMI 1640 containing 10% fetal bovine serum (FBS), 20µm 2-mercaptoethanol, 100 u/ml penicillin, 100 µg/ml streptomycin, and 25 mm HEPES buffer (complete RPMI-1640). Cell count and adjusting cell concentration: This was carried out as per the following procedure. To the cell sample (10µl) trypan blue dye (4%w/v) (10µl) was added to the glass slide and mixed it well. Then cell count was determined using a hemocytometer excluding dead cells. Cell count was adjusted by dilution in complete RPMI-1640 medium to appropriate cell concentration (105 or 5x105 or 1.5x105) as per the assay requirement. In a sterile tissue culture plate, 100 µl of cell suspension was added to each well, and the plate was incubated at 37°C for a minimum of 2 hr under a humidified atmosphere of 95% air and 5% CO_2. Meanwhile, test extracts were prepared in RPMI media containing 10% DMSO maintaining sterility conditions using a 0.22µ filter in the syringe. Different concentrations were prepared in serially diluted manner in a separate 96-well plate as 832µg/ml, 416µg/ml, 208µg/ml, 104µg/ml, 52µg/ml, 26µg/ml, 13µg/ml and 6.5µg/ml. Two hours after the incubation, test extracts/drugs prepared as above were added (20 µl/well) to cultured macrophages and incubated for 24 hr in a humidified incubator containing 5% CO2 at 37ºC. Twenty-four hours after incubation, following assays viz. Sulforhodamine B (SRB) assay, Nitric oxide assay, NBT dye reduction assay, cellular lysosomal enzyme assay, and myeloperoxidase enzyme assay, were performed using (PHA) (Phytohemagglutinin-M) (10 µg/ml) as a positive control.[15, 16]

Measurement of NO production

Nitrite (Nitric oxide) assay on isolated murine peritoneal cells was carried out by following validated laboratory standard operating procedure. Nitric oxide (NO) production was determined by assaying culture supernatants for nitrite using Griess reagent. Isolated murine peritoneal macrophages (5×105 cells/ well) were cultured in complete RPMI 1640, incubated for 2 hr at 37ºC in a 5% CO2 atmosphere. The test extracts dissolved in complete RPMI-1640 containing 10% DMSO were added to cultured wells at various concentrations (832-6.5 μg/ml) in triplicate. The plates were incubated for 24 h at 37ºC in a 5% CO2 atmosphere in CO2 incubator. After 24 h, cell-free supernatant (75 μl) was mixed with 75 μl of Griess reagent (sulfanilamide 1%, phosphoric acid 5%, naphthyl ethylenediamine 0.1%) in another 96 well tissue culture plate and incubated for 10 min at room temperature, protected from light. Cells incubated with PHA (10 μg/ml) were used as a positive control. The optical density (OD) was read at 540 nm using a microplate reader (BioTek). Nitrite concentrations were determined from a standard curve of sodium nitrite in culture conditions. Stimulation index (SI) for NO release was calculated as the nitrite concentrations ratio of the treated and vehicle-treated control cells (RPMI-1640 containing 10% DMSO).[17, 18]

Cellular lysosomal enzyme activity

Cellular Lysosomal enzyme activity assay on isolated murine peritoneal cells was carried out by following validated laboratory standard operating procedure. For lysosomal enzyme assay, isolated murine peritoneal macrophages (1.5×105 cells/ well) were incubated in CO2 incubator for 24 h with the test extracts (832-6.5 μg/ml) in triplicate. For determination of lysosomal enzyme activity, the cultured macrophage monolayers in a 96-well plate were solubilized by the addition of 25 μl of 0.1% Triton X-100. After 15 min incubation at room temperature, 100 μl of 10 mM p-nitrophenyl phosphate was added to a well as a substrate for acid phosphatase. Then 0.1 M citrate buffer (50 μl, pH 5.0) was added to each well and the plate was incubated for 1 h at 37ºC. After incubation, 0.2 M borate buffer (150 μl, pH 9.8) was added to the mixture to stop the reaction. The optical density (OD) was measured at 405 nm by using a microplate reader. Stimulation index (SI) for lysosomal enzyme activity was calculated as the ratio of OD of test extract/ drug-treated cells to vehicle-treated control cells (RPMI-1640 containing 10% DMSO). [19-21]

Nitro blue tetrazolium (NBT) reduction

Nitro Blue Tetrazolium (NBT) reduction assay on isolated murine peritoneal cells was carried out by following validated laboratory standard operating procedure. For NBT dye reduction assay, isolated murine peritoneal macrophages (3×105 cells/well) were incubated in a CO2 incubator at 370C for 24 h with the test extracts/ fractions (832-6.5 μg/ml) in triplicate. The reduction of NBT to insoluble blue formazan was used as a probe for superoxide generation.0.3% NBT (50 μl) solution in RPMI-1640 medium was added to each well of 96 well tissue culture plates. The plate was incubated for 2 hr. After incubation, the supernatants were recovered and the macrophages were fixed by the addition of 200 μl of absolute methanol. Then the plate was washed twice with 70% methanol and then air-dried with the help of a blow of hairdryer. The formazan deposits were solubilized in 120 μl, 2 M KOH, and 140 μl DMSO. After homogenization of the contents in the wells for 10 min., the optical density (OD) was read at 630 nm using a microplate reader (BioTek). The superoxide production was represented as the stimulation index (SI). The stimulation index (SI) of NBT for test extract was calculated as the ratio of OD of test extract to vehicle-treated control cells (RPMI-1640 containing 10% DMSO).[22, 23]

Measurement of myeloperoxidase activity

Myeloperoxidase activity assay on isolated murine peritoneal cells was carried out by following validated laboratory standard operating procedure. For myeloperoxidase activity, macrophages (5 × 105 cells/well) were incubated in CO2 incubator at 370C for 24 h with the test extracts/ fractions (832-6.5 μg/ml) in triplicate. After incubation, cells from each well were washed three times with a fresh RPMI medium. The mixture (100 μl) of o-phenylenediamine (0.4 g/ml) and 0.002% H2O2 in phosphate-citrate buffer (pH 5.0) was added. The reaction was stopped after 10 min using 0.1 N H2SO4. The optical density (OD) was measured at 490 nm using a microplate reader. The myeloperoxidase (MPO) activity was calculated as the stimulation index (SI). The myeloperoxidase stimulation index (SI) was calculated as the ratio of the OD of a test extract-treated well to vehicle-treated control cells (RPMI-1640 containing 10% DMSO.[21, 24]

Statistical Analysis for *in vitro* assays

Results are expressed as mean ± SEM (standard error of the mean) for triplicate assays. Data were analyzed by one-way ANOVA followed by Dunnet's multiple comparisons tests using GraphPad Prism Ver 5 software against control samples. Values of $P<0.05$ were the criteria for statistical significance.

RESULTS AND DISCUSSION

Phytochemical Evaluation of Extracts:

Phytochemical investigations revealed the presence of Alkaloids, Carbohydrates, Flavonoids, Glycosides, Phytosterols, fixed oils and fats, in ethanol extract of *Zingiber capitatum* dried rhizome (ZEE) and Alkaloids, Carbohydrate, Saponins and flavonoids in aqueous extract of *Zingiber capitatum* dried rhizome (ZAE). Results are shown in Table 1.

Table 1: Phytochemical Screening of Extracts of *Zingiber capitatum* dried rhizomes

Phytochemical Test/Reagent	***Zingiber capitatum* Ethanol Extract (ZEE)**	***Zingiber capitatum* Aqueous Extract (ZAE)**
Alkaloids		
Mayer's Test	**+**	+
Dragendroff's Test	**+**	+
Wagner's Test	**+**	+
Carbohydrates		
Molisch's Test	**+**	+
Barford's Test	**-**	-
Benedicts Test	**+**	-
Glycosides		
Molisch's Test after Hydrolysis	**+**	+
Phytosterols		
Liebermann's Burchard's Test	+	+
Fixed Oils And Fats - Spot Test	+	+
Saponins - Foam Test	-	**+**
Phenolic Compounds and Tannins		
Ferric Chloride Test	+	+
Lead Acetate Test	+	-
Proteins and Amino Acids		
Biuret Test	-	-
Ninhydrin Test	-	+
Flavonoids - Shinoda Test	+	+
Yield (%W/W)	5.9%	10%

+ indicates present; - indicates absent

In-vitro Immunomodulatory Activity of *Zingiber capitatum* dried rhizome extract

Zingiber capitatum dried rhizome (ZEE) and aqueous extract of *Zingiber capitatum* dried rhizome (ZAE) were evaluated for *in-vitro* immunomodulatory activity. The extracts of *Zingiber capitatum* were evaluated for release of following immune mediators from murine peritoneal macrophages viz. superoxide (NBT reduction), nitric oxide (NO), lysosomal and myeloperoxidase enzymes.

Measurement of NO production

ZEE extract showed significant stimulation of release of nitric oxide at at 832µg/ml (SI 1.701), 416µg/ml (SI 1.501), 208µg/ml (SI 1.360). (Figure 1) ZAE extracts induced nitrite production in statistically significant P value. ($P<0.05$) at 832µg/ml (SI 2.080),416µg/ml (SI 1.345), 208µg/ml (SI 1.384), 104µg/ml (SI 1.366), 52µg/ml (SI 1.377), 26 µg/ml (SI 1.462), 13µg/ml (SI 1.389), 6.5µg/ml (SI 1.364). (Figure 2)

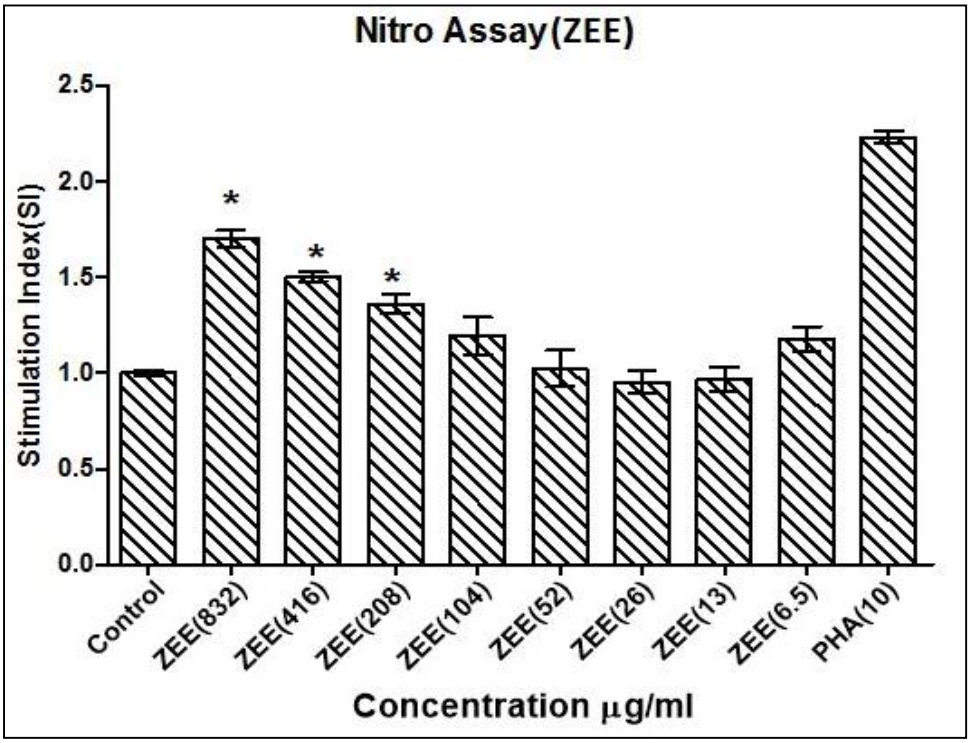

Figure 1: *In-vitro* effect of (ZEE) on release of Nitric Oxide [*significantly different from Control (P < 0.05)]

The nitrite level (Nitric Oxide) produced in cell culture supernatants was measured at 24 hr of treatment, showing that ZEE extract induced nitrite production in statistically significant p-value. ($p<0.05$) at 832µg/ml (SI 1.701), 416µg/ml (SI 1.501), 208µg/ml (SI 1.360). PHA (positive control) also showed significant increase ($p<0.05$) in nitrite release (SI 2.232).

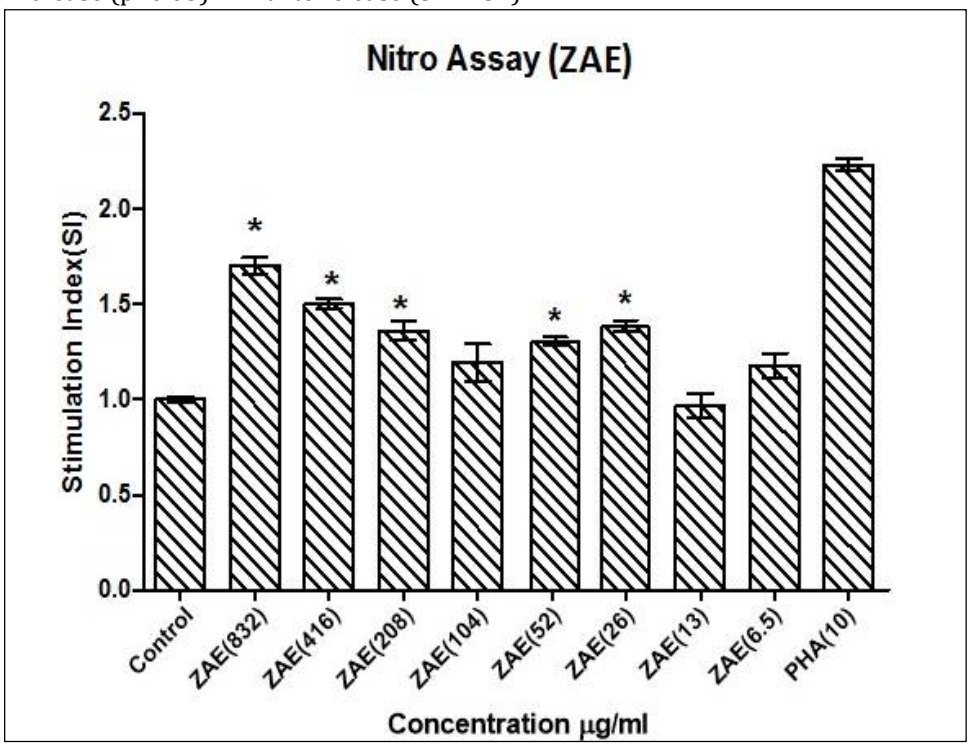

Figure 2: *In-vitro* effect of (ZAE) on release of Nitric Oxide [*significantly different from Control (P < 0.05)]

The nitrite level (Nitric Oxide) produced in cell culture supernatants was measured at 24 hr of treatment, showing that ZAE extract induced nitrite production in statistically significant p-value. ($p<0.05$) at 832µg/ml (SI 1.701), 416µg/ml (SI 1.501), 208µg/ml (SI 1.360), 52µg/ml (SI 1.377), 26 µg/ml (SI 1.462). PHA (positive control) also showed significant increase ($p<0.05$) in nitrite release (SI 2.232).

Cellular lysosomal enzyme activity

ZEE extract showed significant stimulation of lysosomal enzyme activity at 832µg/ml (SI 1.558), 416µg/ml (SI 1.345), 208 µg/ml (SI 1.259). (Figure 3) In case of lysosomal enzyme activity ZAE extract showed at 832µg/ml (SI 1.606), 416µg/ml (SI 1.281), 208µg/ml (SI 1.157), 26 µg/ml (SI 1.154). (Figure 4)

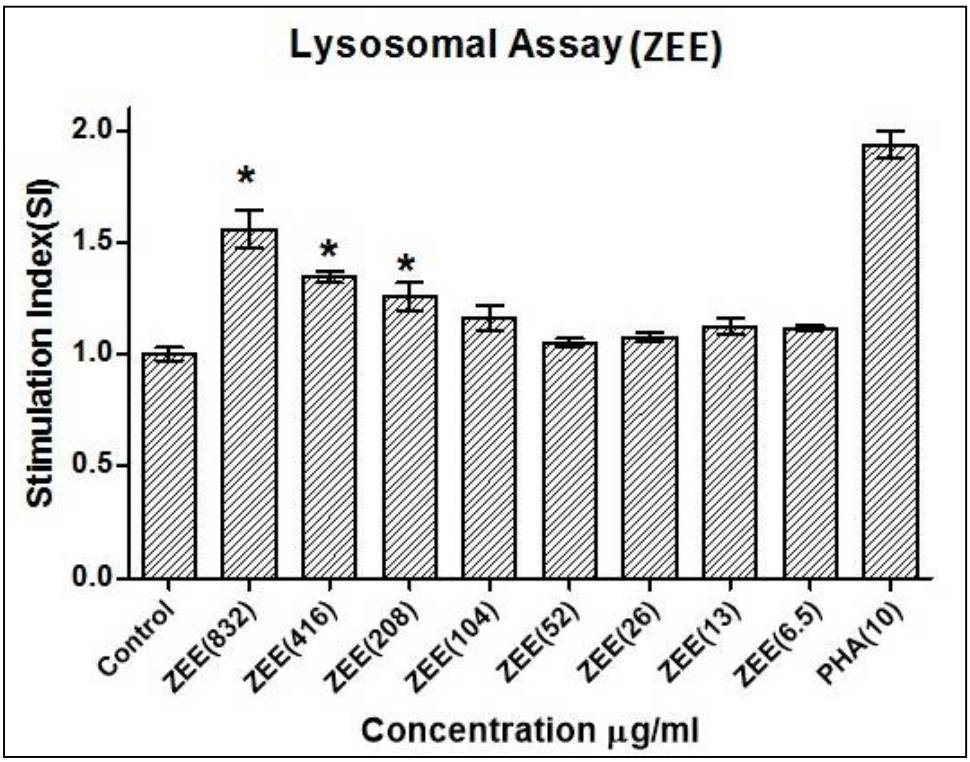

Figure 3: *In-vitro* effect of (ZEE) on Cellular Lysosomal activity assay[*Significantly different from Control (P < 0.05)]

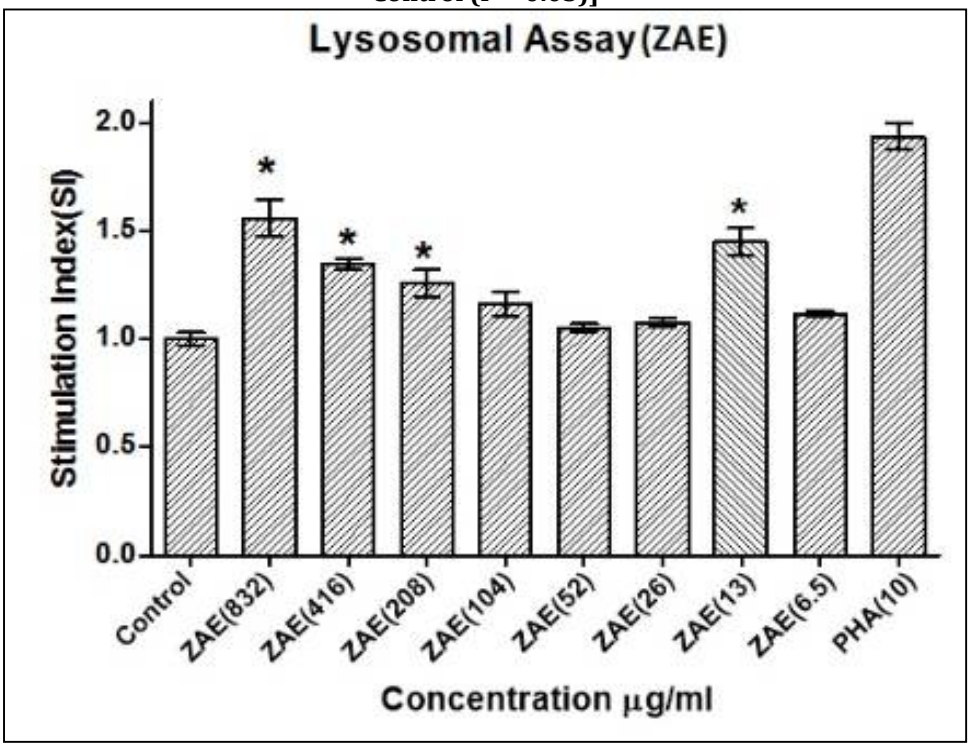

Figure 4: *In-vitro* effect of (ZAE) on Cellular Lysosomal activity assay [*Significantly different from Control (P < 0.05)]

In case of Lysosomal enzyme activity ZEE extract showed at 832µg/ml (SI 1.558), 416µg/ml (SI 1.345), 208µg/ml (SI 1.259). In case of Lysosomal enzyme activity ZAE extract showed at 832µg/ml (SI 1.558),

416µg/ml (SI 1.345), 208µg/ml (SI 1.259), 13µg/ml (SI 1.462). PHA showed significant stimulation ($p<0.05$) of lysosomal enzyme release (SI 1.937) for both extract.

Nitro blue tetrazolium (NBT) reduction

ZEE extract showed significant stimulation in NBT reduction at 832µg/ml (SI 1.489), 416µg/ml (SI 1.393), 208µg/ml (SI 1.315), 104µg/ml (SI 1.276), 52µg/ml (SI 1.245), 26 µg/ml (SI 1.174), 13 µg/ml (SI 1.184), 6.4µg/ml (SI 1.013). (Figure 5) The effect of ZAE extract showed significant stimulation ($P<0.05$) on NBT reduction at 832µg/ml (SI 1.588), 416µg/ml (SI 1.350), 208µg/ml (SI 1.367), 104µg/ml (SI 1.225), 52µg/ml (SI 1.315), 26 µg/ml (SI 1.187) & 13 µg/ml (SI 1.226). (Figure 6)

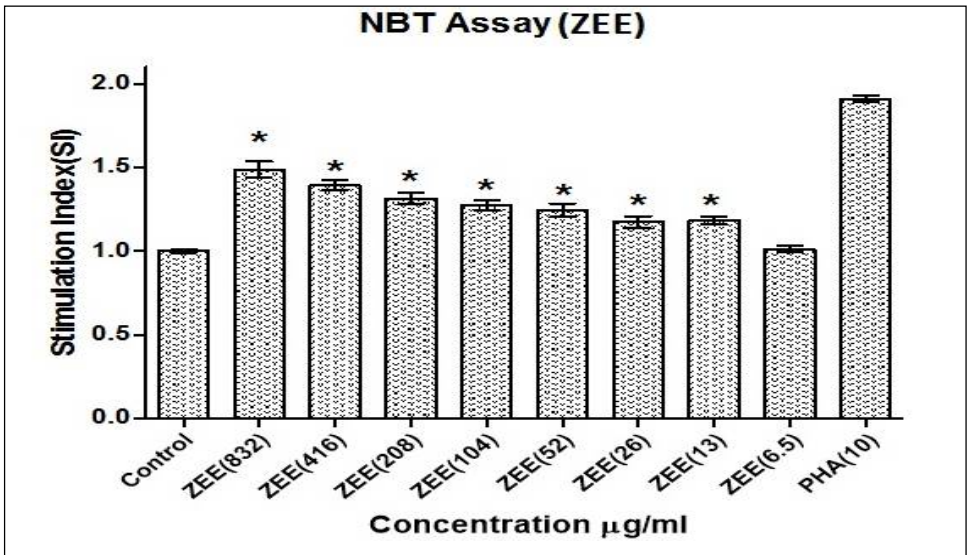

Figure 5: *In-vitro* effect of (ZEE) on NBT dye reduction activity [*significantly different from Control (P < 0.05)]

The *in-vitro* phagocytic effects of different concentrations of ZEE extract on the reduction of NBT dye on macrophages are presented in figure The effect of ZEE extract showed significant stimulation ($p<0.05$) on NBT reduction at 832µg/ml (SI 1.489) , 416µg/ml (SI 1.393), 208µg/ml (SI 1.315), 104µg/ml (SI 1.276), 52µg/ml (SI 1.245),26 µg/ml (SI 1.174),13 µg/ml (SI 1.184), 6.4µg/ml (SI 1.013), PHA showed significant stimulation ($p<0.05$) of NBT reduction (SI 1.910).

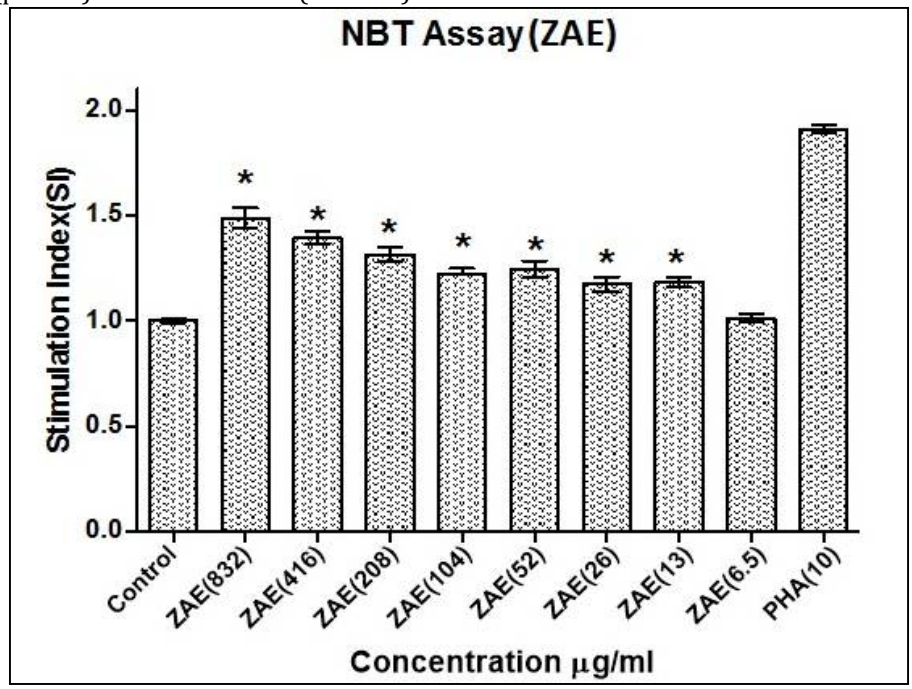

Figure 6: *In-vitro* effect of (ZAE) on release of NBT dye reduction activity [*significantly different from Control (P < 0.05)]

The *in vitro* phagocytic effects of different concentrations of ZAE extract on the reduction of NBT dye on macrophages are presented in figure The effect of ZAE extract showed significant stimulation ($p<0.05$) on

NBT reduction at 832µg/ml (SI 1.489) , 416µg/ml (SI 1.393), 208µg/ml (SI 1.315), 104µg/ml (SI 1.276), 52µg/ml (SI 1.245),26 µg/ml (SI 1.174),13 µg/ml (SI 1.184), 6.4µg/ml (SI 1.013), PHA showed significant stimulation ($p<0.05$) of NBT reduction (SI 1.910).

Measurement of myeloperoxidase activity

ZEE extract showed significant effect on myeloperoxidase activity as compared to control wells at 832µg/ml (SI 1.640), 416µg/ml (SI 1.483), 208µg/ml (SI 1.326), 104µg/ml (SI 1.229) & 26µg/ml (SI 1.182). (Figure 7). The ZAE extract showed significant ($P<0.05$) stimulation of myeloperoxidase activity of macrophages at at 832µg/ml (SI 1.656), 416µg/ml (SI 1.320), 208µg/ml (SI 1.192), 52µg/ml (SI 1.132), 26 µg/ml (SI 1.188), 13µg/ml(SI 1.129), 6.5 µg/ml (SI 1.135). (Figure 8)

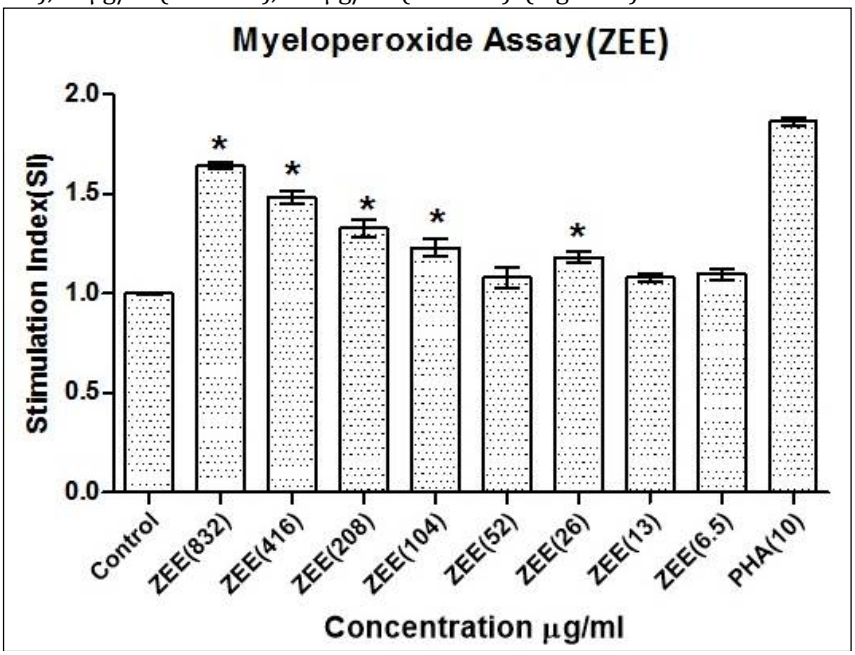

Figure 7: *In-vitro* effect of (ZEE) on Cellular Myeloperoxidase activity assay [*significantly different from Control (P < 0.05)]

The effect of ZEE extract on myeloperoxidase activity of macrophages is presented in figure. The ZEE extract showed significant ($p<0.05$) stimulation of myeloperoxidase activity of macrophages at 832µg/ml (SI 1.640), 416µg/ml (SI 1.483), 208µg/ml (SI 1.326), 104µg/ml (SI 1.229) & 26µg/ml (SI 1.182). Positive control, PHA showed significant stimulation with SI value (1.862).

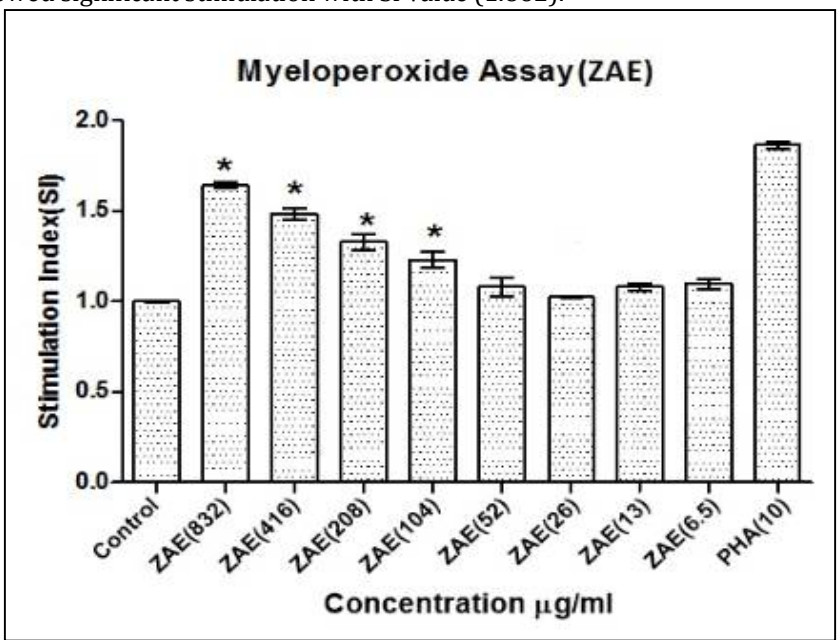

Figure 8: *In-vitro* effect of (ZAE) on Cellular Myeloperoxidase activity assay [*significantly different from Control (P < 0.05)]

The effect of ZAE extract on myeloperoxidase activity of macrophages is presented in figure. The ZAE extract showed significant ($p<0.05$) stimulation of myeloperoxidase activity of macrophages at 832µg/ml (SI 1.640), 416µg/ml (SI 1.483), 208µg/ml (SI 1.326), 104µg/ml (SI 1.229). Positive control, PHA showed significant stimulation with SI value (1.862).

CONCLUSION:

An increase in nitrite and superoxide production has a significant effect on the function of macrophages which subsequently increases the cytotoxic activity of the macrophages. Effects of different concentrations of the extract on the superoxide generation in terms of the reduction of NBT dye and nitrite release in culture supernatant were demonstrated. Myeloperoxidase produces hypochlorous acid (HOCl) and hydrogen peroxide to exhibit a cytotoxic effect. In the present study, the extract showed a maximum increase in enzyme activity. Study concluded that ethanol aqueous extract of *Zingiber capitatum* Roxbrhizome stimulates non-specific immune responses by stimulating macrophages. *In-vivo* studies also need to be done to correlate with the *in-vitro* results.

AUTHORS CONTRIBUTION STATEMENT:

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CONFLICT OF INTEREST:

Conflict of interest declared none.

REFERENCES

1. Kaufman, S. (2016). Zingiber capitatum (wild ginger). *Invasive Species Compendium*, (121135).
2. Ravindra Zode, WalayTagade and Mahesh Meshram. *Zingiber Capitatum* Roxb - A New Report for Gondia District, (MS) India. In book: Current Updates in Life Sciences, Publisher: Pandit Jawaharlal Nehru study center, Shiwaji Science College, Akola (MS) India. pp. 302-307.
3. Jha, R. R. and Verma, S. K. (1995) Critical account of *Zingiberaceae* of Bihar : I Genus *Zingiber Bochmen*. In. A.K. Pandey, Taxonomy and Biodiversity. CBS . Delhi. Pages 108-115.
4. Jena, A. K., Sahoo, R. K., Subudhi, E., Ghosh, G., Subudhi, B. B., & Nayak, S. (2011). Studies on antioxidant, antimicrobial and phytochemical analysis of Zingiber capitatum roxb. Rhizome extracts. *Int. J. Integr. Biol*, *11*, 127-133.
5. Kulkarni, D. K., Bhusare, S., Kasarkar, A. R., & Sabu, M. (2013). New Report of zingiber capitatum Roxb from Mendha Lekha, Maharashtra. *Science Research Reporter*, *3*(1), 49-52.
6. Cooper MD, Schroeder HW. Primary immune deficiency diseases. In: Harrison TR, editor. *Harrison's Principles of Internal Medicine.* 16. New York: Mc Graw-Hill; 2005. pp. 1939–41.
7. Nfambi, J., Bbosa, G. S., Sembajwe, L. F., Gakunga, J., & Kasolo, J. N. (2015). Immunomodulatory activity of methanolic leaf extract of Moringa oleifera in Wistar albino rats. *Journal of basic and clinical physiology and pharmacology*, *26*(6), 603-611.
8. Chan M. *Address at the WHO Congress on Traditional Medicine.* WHO; 2008. Available at: http://www.who.int/dg/speeches/2008/20081107/en/
9. Dahanukar, S. A., Kulkarni, R. A., & Rege, N. N. (2000). Pharmacology of medicinal plants and natural products. *Indian journal of pharmacology*, *32*(4), S81-S118.
10. Gupta, A., Verma, S., Gupta, A., Jangra, M., & Pratap, R. (2015). Evaluation of Prosopis cineraria (Linn.) Druce leaves for wound healing activity in rats. *Ann. Pharm. Res*, *3*, 70-74.
11. Trease GE, Evans MS. A Textbook of Pharmacognosy. In: Evans WC, Ed. 14, W.B. Saunders Company Ltd., London; 1999: 229.
12. Khandelwal KR. Practical pharmacognosy, techniques and experiments. 17th ed. Pune: Nirali Prakashan Publishers; 2007, p. 149-156.
13. Soni, L. K., Parasher, P., & Dobhal, M. P. (2017). Phytochemical Evaluation of Prosopis cineraria, Curcuma amada and Citrulluscolocynthis. *Journal of Environment, Science and Technology*, *3*(2), 42-49.

14. Pathak, V., & Kumar, P. (2017). Phytochemical Screening of Prosopis cineraria (L.) Stem Bark and Leaves. *International Journal of Innovative Science and Research Technology*, *2*(8), 306-317.
15. Chaves, F., Chacón, M., Badilla, B., & Arévalo, C. (2007). Effect of Echinacea purpurea (Asteraceae) aqueous extract on antibody response to Bothrops asper venom and immune cell response. *Revista de biología tropical*, *55*(1), 113-119.
16. Djafoua, Y. M., Mouokeu, R. S., Tume, C., Kamtchueng, M. O., & Kuiate, J. R. (2015). Immunomodulatory activity of methanol leaf extracts of Cameroonian medicinal plants. *Journal of Complementary and Integrative Medicine*, *12*(4), 267-275.
17. Lee, S., & Suk, K. (2007). Heme oxygenase-1 mediates cytoprotective effects of immunostimulation in microglia. *Biochemical pharmacology*, *74*(5), 723-729.
18. Keller, R., Keist, R., Wechsler, A., Leist, T. P., & Van der Meide, P. H. (1990). Mechanisms of macrophage-mediated tumor cell killing: a comparative analysis of the roles of reactive nitrogen intermediates and tumor necrosis factor. *International journal of cancer*, *46*(4), 682-686.
19. Sunil, M. A., Sunitha, V. S., Radhakrishnan, E. K., & Jyothis, M. (2019). Immunomodulatory activities of Acacia catechu, a traditional thirst quencher of South India. *Journal of Ayurveda and Integrative Medicine*, *10*(3), 185-191.
20. Suzuki, I., Tanaka, H., Adachi, Y., & Yadomae, T. (1988). Rapid measurement of phagocytosis by macrophages. *Chemical and Pharmaceutical Bulletin*, *36*(12), 4871-4875.
21. Shah, A. S., & Juvekar, A. R. (2010). *In vitro* and in vivo immunostimulatory activity of Woodfordia fruticosa flowers on non-specific immunity. *Pharmaceutical biology*, *48*(9), 1066-1072.
22. Heroor, S., Beknal, A. K., & Mahurkar, N. (2013). Immunomodulatory activity of methanolic extracts of fruits and bark of Ficus glomerata Roxb. in mice and on human neutrophils. *Indian journal of pharmacology*, *45*(2), 130.
23. Rainard, P. (1986). A colorimetric microassay for opsonins by reduction of NBT in phagocytosing bovine polymorphs. *Journal of Immunological Methods*, *90*(2), 197-201.
24. Pinegin, B. V., Butakov, A. A., & Shelcina, T. L. (1995). Complex of methods for determination of functional activity of phagocyting cells. In *Ecological immunology*. VNIRO Moscow, 146-154.

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