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Exploring the Bioactivity of Alpinia Calcarata: Ethanolic Rhizome Extract-Based Pharmacological Studies

Dr. Nagesh S. Tour[1], Mr. Venkat G. Chougule[2], Dr. Gitanjali C. Chavan[3], Ms. Renuka J. Shinde[4], Mr. Vishal G. Sakhare[5], Miss. Pratima B. Ovhal[6], Dr. Mangesh G. Bhosale[7]
[1]Professor, ASPM's K. T. Patil College of Pharmacy, Dharashiv
[2]Assistant Professor, ASPM's K. T. Patil College of Pharmacy, Dharashiv.
[3,5]Assistant Professor, SBSPMs B. Pharmacy College Ambajogai Dist.Beed.
[4]Assistant Professor, SVERI's College of Pharmacy, Pandharpur.
[6]Vitthal Pratishthan College of Pharmacy Madha, Dist-Solapur.
[7]Assistant Professor, Department of Chemistry, Ramkrishna Paramhansa Mahavidyalaya, Dharashiv

ABSTRACT
The present study investigates the pharmacological potential of the ethanolic extract of Alpinia calcarata rhizome, a traditional Ayurvedic herb, focusing on its anti-asthmatic, antioxidant, and anti-inflammatory activities. Phytochemical screening confirmed the presence of flavonoids, alkaloids, phenolics, and terpenoids. The anti-asthmatic effect was assessed using animal models including histamine-induced bronchospasm and acetylcholine-induced bronchoconstriction in guinea pigs. Antioxidant activity was evaluated by DPPH and ABTS radical scavenging assays, while anti-inflammatory potential was tested using carrageenan-induced paw edema in rats. The extract showed significant bronchodilation, free radical scavenging activity, and inhibition of inflammation in a dose-dependent manner. These findings support the traditional use of Alpinia calcarata and suggest its potential as a natural therapeutic agent in asthma and inflammation management.
Keywords: *Alpinia calcarata, Anti-asthmatic, Antioxidant, Anti-inflammatory, Ethanolic extract, Bronchodilation and Oxidative stress.*

1.INTRODUCTION

The term "**medicinal plant**" includes various types of plants used in herbalism ("herbology" or "herbal medicine"). It is the use of plants for medicinal purposes, and the study of such uses. The word "**herb**" has been derived from the Latin word, "herba" and an old French word "herbe". Now days, herb refers to any part of the plant like fruit, seed, stem, bark, flower, leaf, stigma or a root, as well as a non- woody plant. Earlier, the term "herb" was only applied to non-woody plants, including those that come from trees and shrubs. These medicinal plants are also used as food, flavonoid, medicine or perfume and also in certain spiritual activities.

Plants have been used for medicinal purposes long before prehistoric period. Ancient Unani manuscripts Egyptian papyrus and Chinese writings described the use of herbs. Evidence exist that Unani Hakims, Indian Vaids and European and Mediterranean cultures were using herbs for over 4000 years as medicine. Indigenous cultures such as Rome, Egypt, Iran, Africa and America used herbs in their healing rituals, while other developed traditional medical systems such as Unani, Ayurveda and Chinese Medicine in which herbal therapies were used systematically.

Among ancient civilizations, India has been known to be rich repository of medicinal plants. The forest in India is the principal repository of large number of medicinal and aromatic plants, which are largely collected as raw materials for manufacture of drugs and perfumery products. About 8,000 herbal remedies have been codified in AYUSH systems in INDIA. Ayurveda, Unani, Siddha and Folk (tribal) medicines are the major systems of indigenous medicines. Among these systems, Ayurveda and Unani Medicine are most developed and widely practiced in India.

Recently, WHO (World Health Organization) estimated that 80 percent of people worldwide rely on herbal medicines for some aspect of their primary health care needs. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants.

Treatment with medicinal plants is considered very safe as there is no or minimal side effects. These remedies are in sync with nature, which is the biggest advantage. The golden fact is that, use of herbal treatments is independent of any age groups and the sexes.

The ancient scholars only believed that herbs are only solutions to cure a number of health related problems and diseases. They conducted thorough study about the same, experimented to arrive at

accurate conclusions about the efficacy of different herbs that have medicinal value. Most of the drugs, thus formulated, are free of side effects or reactions. This is the reason why herbal treatment is growing in popularity across the globe. These herbs that have medicinal quality provide rational means for the treatment of many internal diseases, which are otherwise considered difficult to cure.

Importance of some herbs with their medicinal values:

- Herbs such as black pepper, cinnamon, myrrh, aloe, sandalwood, ginseng, red clover, burdock, bayberry, and safflower are used to heal wounds, sores and boils.
- Basil, Fennel, Chives, Cilantro, Apple Mint, Thyme, Golden Oregano, Variegated Lemon Balm, Rosemary, Variegated Sage are some important medicinal herbs and can be planted in kitchen garden. These herbs are easy to grow, look good, taste and smell amazing and many of them are magnets for bees and butterflies.
- Many herbs are used as blood purifiers to alter or change a long-standing condition by eliminating the metabolic toxins. These are also known as 'blood cleansers'. Certain herbs improve the immunity of the person, thereby reducing conditions such as fever.
- Some herbs are also having antibiotic properties. Turmeric is useful in inhibiting the growth of germs, harmful microbes and bacteria. Turmeric is widely used as a home remedy to heal cut and wounds.
- To reduce fever and the production of heat caused by the condition, certain antipyretic herbs such as Chirayta, black pepper, sandal wood and safflower are recommended by traditional Indian medicine practitioners.
- Sandalwood and Cinnamon are great astringents apart from being aromatic. Sandalwood is especially used in arresting the discharge of blood, mucus etc.
- Some herbs are used to neutralize the acid produced by the stomach. Herbs such as marshmallow root and leaf. They serve as antacids. The healthy gastric acid needed for proper digestion is retained by such herbs.
- Indian sages were known to have remedies from plants which act against poisons from animals and snake bites.
- Herbs like Cardamom and Coriander are renowned for their appetizing qualities. Other aromatic herbs such as peppermint, cloves and turmeric add a pleasant aroma to the food, thereby increasing the taste of the meal.

ASTHMA:

Asthma is a chronic inflammatory lung disease that can cause repeated episodes of cough, wheezing and breathing difficulty.

During an acute asthma episode, the airway lining in the lungs becomes inflamed and swollen. In addition, mucus production occurs in the airway and muscles surrounding the airway spasm. Combined, these cause a reduction in air flow. [1]

Asthma is characterized by:

Airway inflammation: The airway lining becomes red, swollen, and narrow.

Airway obstruction: The muscles encircling the airway tighten causing the airway to narrow making it difficult to get air in and out of the lungs.

Airway hyper-responsiveness: The muscles encircling the airway respond more quickly and vigorously to small amounts of allergens and irritants.

Inflammatory mediators:

Chemokines are important in recruitment of inflammatory cells into the airways and are mainly expressed in airway epithelial cells. Eotaxin is relatively selective for eosinophils, whereas thymus and activation-regulated chemokines (TARCs) and macrophage-derived chemokines (MDCs) recruit Th2 cells. There is an increasing appreciation for the role this family of mediators has in orchestrating injury, repair, and many aspects of asthma.

Cytokines direct and modify the inflammatory response in asthma and likely determine its severity. Th2-derived cytokines include IL-5, which is needed for eosinophil differentiation and survival, and IL-4 which is important for Th2 cell differentiation and with IL-13 is important for IgE formation. Key cytokines include IL-1β and tumor necrosis factor-α (TNF-α), which amplify the inflammatory response, and granulocyte-macrophage colony-stimulating factor (GM-CSF), which prolongs eosinophil survival in airways. Recent studies of treatments directed toward single cytokines (e.g., monoclonal antibodies against IL-5 or soluble IL-4 receptor) have not shown benefits in improving asthma outcomes.

Cysteinyl-leukotrienes are potent bronchoconstrictors derived mainly from mast cells. They are the only mediator whose inhibition has been specifically associated with an improvement in lung function and asthma symptoms. Recent studies have also shown leukotriene B_4 can contribute to the inflammatory process by recruitment of neutrophils.

Nitric oxide (NO) is produced predominantly from the action of inducible NO synthase in airway epithelial cells; it is a potent vasodilator. Measurements of fractional exhaled NO(FeNO) may be useful for monitoring response to asthma treatment because of the purported association between FeNO and the presence of inflammation in asthma).

Immunoglobulin E (IgE) is the antibody responsible for activation of allergic reactions and is important to the pathogenesis of allergic diseases and the development and persistence of inflammation. IgE attaches to cell surfaces via a specific high-affinity receptor. The mast cell has large numbers of IgE receptors; these, when activated by interaction with antigen, release a wide variety of mediators to initiate acute bronchospasm and also to release pro-inflammatory cytokines to perpetuate underlying airway inflammation.

INFLAMMATION:

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. It is a body defense reaction in order to eliminate or limit the spread of injurious agent.

Causes of inflammation;

Infective agents. E.g.: Bacteria, viruses and their toxins.
Immunological agents. E.g.: Cell mediated and antigen antibody reaction.
Physical agents. E.g.: Heat, cold, radiation, mechanical trauma.
Chemical agents. E.g.: Organic and inorganic poisons

Granulomatous inflammation:

Granuloma is defined as the tiny lesion about 1 mm diameter, composed predominantly of collection of modified macrophages called epithelioid cells and rimmed at periphery by lymphoid cells.

Examples of granulomatous Inflammation-Tuberculosis, leprosy, fungal infections.

ANTIOXIDANTS:

The ability to utilize oxygen has provided humans with the benefit of metabolizing fats, proteins, and carbohydrates for energy; however, it does not come without cost. Oxygen is a highly reactive atom that is capable of becoming part of potentially damaging molecules commonly called “free radicals.” Free radicals are capable of attacking the healthy cells of the body, causing them to lose their structure and function.

Cell damage caused by free radicals appears to be a major contributor to aging and to degenerative diseases of aging such as cancer, cardiovascular disease, cataracts, immune system decline, and brain dysfunction. Overall, free radicals have been implicated in the pathogenesis of at least 50 diseases. Fortunately, free radical formation is controlled naturally by various beneficial compounds known as antioxidants. It is when the availability of antioxidants is limited that this damage can become cumulative and debilitating.

PLANT PROFILE:

Figure: Alpinia calcarata – PLANT

Figure No.2: Rhizome.

Preliminary Phytochemical Screening of Ethanolic Extract of Alpinia Calcarata Rhizomes (Eeac):

The phytochemical screening of the ethanolic extract of the Alpinia calcarata rhizomes indicate the presence of carbohydrate, cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins and phenolic compounds.

Table: Preliminary phytochemical analysis

(+: presence, -: absence)

Sl. No:	Constituents	Presence/absence
1	Carbohydrate	+
2	Proteins	+
3	Amino acids	-
4	Fats and oils	-
5	Steroids	+
6	Cardiac glycosides	+
7	Anthraquinone glycoside	-
8	Saponin glycosides	-
9	Cyanogenic glycosides	-
10	Coumarin glycosides	-
11	Flavonoids	+
12	Alkaloids	-
13	Tannins	+
14	Phenol	+

METHODS:

Collection and authentication of Alpinia calcarata:

The dried rhizomes of the Alpinia calcarata were collected. The rhizomes were cleaned and shade dried and milled into coarse powder by a mechanical grinder.

Preparation of plant extract

The powdered rhizomes were extracted using ethanol by soxhlet extractor. In this process the powdered drug is placed into the extractor with ethanol as solvent. After extraction the extract was concentrated by evaporation then it was kept in a refrigerator for further use.

ACUTE TOXICITY STUDIES:

Acute toxicity of Alpinia calcarata was done as per OECD guidelines 423. The substance was administered in a single dose by gavage using specially designed mice oral tube. Animals were fasted prior to dosing with food but not water withheld overnight. Following the period of fasting, the animals were weighed and the test substance was orally at a dose of 5, 50, 300 and 2000 mg/kg. The animals are observed continuously for first three hours, four any toxic manifestations like increased motor activity, salivation, acute convulsion, coma and death. Changes in the animal behavior should be noted before and after administration for 24hours. Treated animals are to be further observed for 14 days. If the extract does

not produce mortality at the highest dose, then the 1/10th or 1/20th of the dose was selected for experiment.

In vivo anti-asthmatic activity:

Histamine aerosol induced bronchoconstriction in guinea pigs:

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by a nebulizer in an aerosol chamber. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2% histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received carboxy methyl cellulose. The animals of group 2 and 3 were given, by oral intubation, 100 and 200 mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hours, to determine pre convulsive time (PCT).

Percentage protection was calculated using the formula.

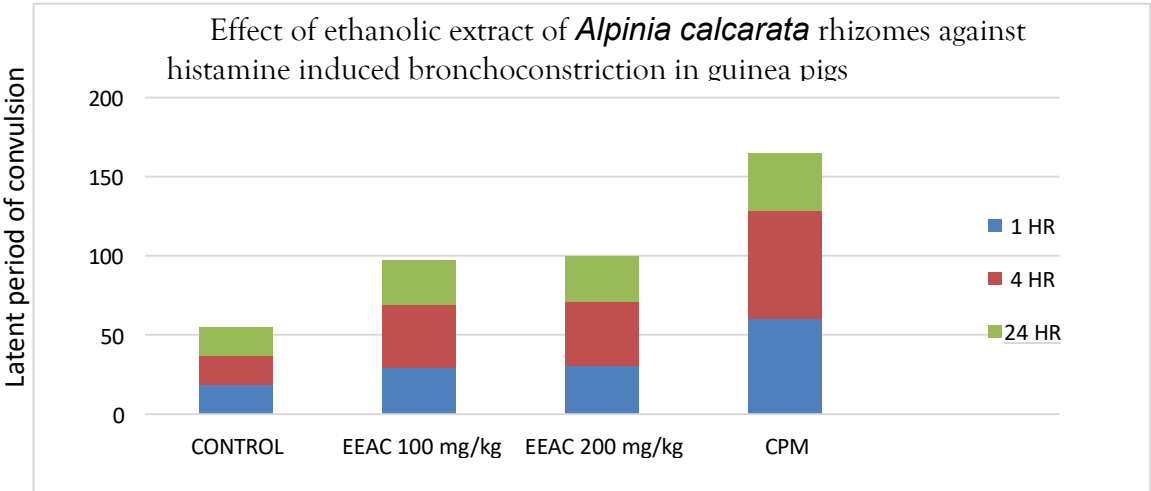
$$\text{Percentage protection} = \frac{Eta - Etb}{Eta} \times 100$$

Where Eta is the preconvulsion time after administration of drug and Etb is the preconvulsion time before administration of drug.

Table: Histamine aerosol induced bronchoconstriction in guinea pigs

Group	Latent period of convulsion			
	Before	1 hour	4 hour	24 hour
Control	16.3±2.23	18.36±0.183	18.63±0.186	18.4±0.12
Alpinia calcarata Ethanolic extract (100 mg/kg)	16.71±1.31	29.65±.28	39.38±0.05*	28.2±0.23
Alpinia calcarata Ethanolic extract (200 mg/kg)	15.71±0.77	30.5±3.08	40.36±1.04*	28.4±.35
Standard (CPM) (1 mg/kg)	18.46±0.89	60.25±0.03*	68.26±1.01**	36.5±0.55

Figure: Effect of ethanolic extract of Alpinia calcarata rhizomes against histamine induced bronchoconstriction in guinea pig.



IN VITRO ANTIOXIDANT ACTIVITY

Hydrogen peroxide scavenging

Hydrogen peroxide solution (20 Mm) was prepared with standard phosphate buffer (pH 7.4). Extract samples (25, 50, 100, 200 and 400 µg/ml) in distilled water were added to hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the reference standard. The percentage scavenging of hydrogen peroxide of plant extract was calculated using the formula.[55]

$$\% \text{ Scavenged} = \frac{Ac - As}{Ac} \times 100$$

Where, Ac = Absorbance of control

As = Absorbance of sample

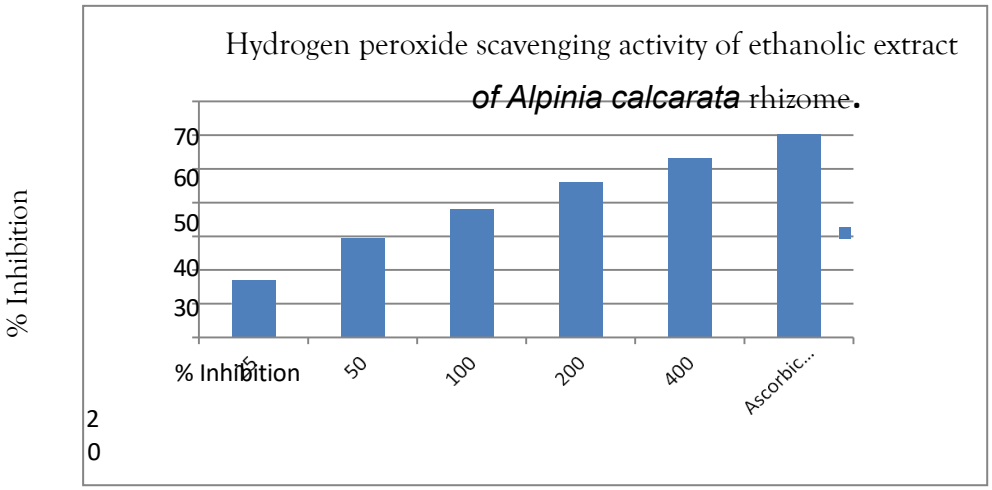
The experiments were performed in triplicates, and the results were expressed as Mean± S.E.M

Table : Hydrogen peroxide scavenging activity of ethanolic extract of Alpinia calcarata rhizomes.

Sl no	Concentration(µg/ml)	Absorbance [A]	% inhibition
1	25	0.632±0.0005	17.16
2	50	0.539±0.0052	29.5
3	100	0.474±0.0056	38.04
4	200	0.414±0.0005	46
5	400	0.357±0.0032	53.3
6	Ascorbic acid (100 µg/ml)	0.256±0.056	60.23

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 **(significant) compared with control. Statistical analysis was done by one-way analysis of variance followed by Dunnett's multiple comparison test.

Figure: Hydrogen peroxide scavenging activity of ethanolic extract of Alpinia calcarata rhizome.



IN VITRO ANTI-INFLAMMATORY ACTIVITY:

Protein denaturation:

A solution of 0.2% of bovine serum albumin (BSA) was prepared in tris buffer saline and pH was adjusted to 6.8 using glacial acetic acid. Test drug of different concentration (25, 50, 100, 200 and 400 µg/ml) was prepared using ethanol as solvent. 50 µl of each test drug was transformed to test tubes using micropipette. 5 ml of 0.2% w/v of BSA was added to the test tubes. The control consists of 5 ml of 0.2% w/v of BSA solution and 5µl alcohol. The test tubes were heated at 72^0 C for 5 min and then cooled

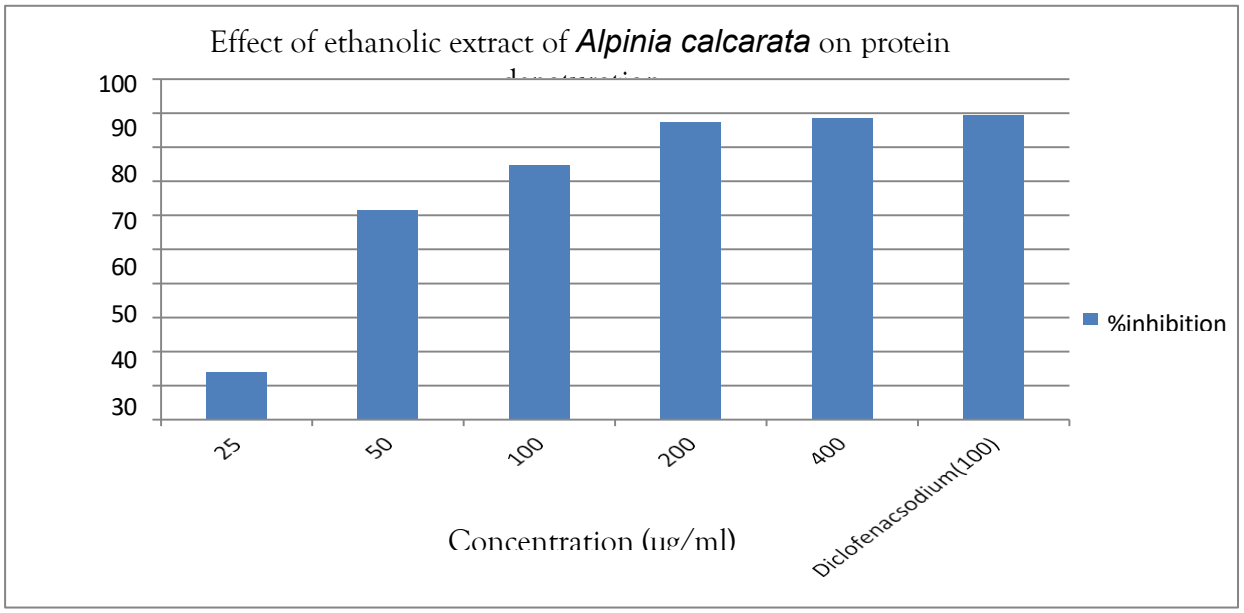
for 10 min. The absorbance of these solution was determined using UV–visible spectrophotometer at 660nm. Diclofenac sodium was used as standard and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated using the following formula.

Table: Effect of ethanolic extract of Alpinia calcarata on protein denaturation.

Sl no	Concentration (µg/ml)	Absorbance [A]	% inhibition
1	25	1.28±0.05	14
2	50	0.578±0.03	61.6
3	100	0.382±0.002	74.63
4	200	0.189±0.01	87.4
5	400	0.172±0.002	88.57
6	Diclofenac sodium (100µg/ml)	0.165±0.005	89.43

(Values are Mean±S.E.M., where n=6) in each group, P< 0.05 *, P< 0.01 ** (significant) compared with control. Statistical analysis was done by one-way analysis of variance followed by Dunnett's multiple comparison test.

Figure: Effect of ethanolic extract of Alpinia calcarata on protein denaturation.



DISCUSSION:

Histamine is one of the major inflammatory mediators in the immediate phase of asthma, causing airway hyper responsiveness and bronchial airway inflammation. Histamine induced bronchodialators is the traditional immunological model of antigen induced airway obstruction. Histamine when inhaled causes hypoxia and leads to convulsion in guinea pigs and causes very strong smooth muscle contraction, profound hypotension and capillary dialation in the cardiovascular system. A prominent effect caused by histamine leads to severe bronchoconstriction in the guinea pigs that causes convulsion and leads to death. Bronchodialators can delay the occurrence of these symptoms. In this histamine induced bronchospasm the ethanolic extract of the plant Alpinia calcarata rhizomes showed significant activity and increase in dose of extract increase % protection. The maximum % protection shown by the plant extract was 60.79 % observed at 200 mg/kg for bronchorelaxant study comparable to that of standard drug chlorpheniramine maleate 78.3 %.

The results of the study confirmed the bronchodialotor properties of the plant, justifying its traditional claim in the treatment of asthma.

The ethanolic extract of Alpinia calcarata rhizomes showed good dose dependent hydrogen peroxide scavenging activity. Hydrogen peroxide (H_2O_2), a biologically relevant, non- radical oxidizing species, may

be formed in tissue through oxidative processes. Although hydrogen peroxide is a weak oxidizing agent it can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. Hydrogen peroxide itself is not reactive, but can generate hydroxyl radical (OH) (via fenton reaction) in the cells resulting in initiation and propagation of lipid peroxidation. Thus the removal of H_2O_2 is very important for antioxidant defence in cell or food systems. Hydrogen peroxide can cross cell membranes and may oxidize a number of compounds. The ability of the extracts to quench OH^- seems to be directly related to the prevention of the lipid peroxidation and appears to be moderate scavenger of active oxygen species, thus reducing rate of chain reaction.

As a conclusion the ethanolic extract of the Alpinia calcarata rhizomes showed hydrogen peroxide activity as compared to standard ascorbic acid (100 µg/ml).

Denaturation of protein is a well-documented cause of inflammation. Production of auto antigens may be due to the denaturation of tissue protein. Agents that can prevent protein denaturation therefore would be worthwhile for anti- inflammatory drug development. The mechanism of denaturation probably involves the alteration electrostatic hydrogen, hydrophobic and disulphide bonding. It has been reported that one of the features of several non-steroidal anti-inflammatory drugs in their ability to stabilize heat treated protein at physiological pH.

The ethanolic extract of the Alpinia calcarata rhizomes exhibited concentration dependant inhibition of protein denaturation. Therefore, from the study it can be concluded that the rhizomes of the plant extract possess marked in vitro anti- inflammatory effect.

CONCLUSION:

The result of the investigation showed that the ethanolic extract of Alpinia calcarata rhizomes possess anti asthmatic activity. The antioxidant and anti- inflammatory property of the plant also supports its anti-asthmatic property. Drugs effective in asthma are mostly steroidal in nature. Phytochemical analysis showed presence of flavonoid and steroids. The anti-asthmatic property showed by the plant may be because of these chemical moieties. The results obtained in the study supports the traditional and also demands further research and to isolate and characterize active principles responsible for anti-asthmatic activity.

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