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Pharmacological Assessment of The Ethanolic extract of Alpinia Calcarata Rhizome For Anti-Inflammatory and Antioxidant Activities

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ABSTRACT

To evaluate the antibacterial activity and chemical constituents of the essential oil from the Artemisia argyi grown in Qichun (China). Alpinia calcarata rhizome ethanolic extract was tested for anti-asthmatic, antioxidant, and anti-inflammatory properties. Adaptogens normalize leukocytosis after milk consumption. Eosinophils are necessary for allergic illness development. The plant extract significantly reduced allergic asthmarelated eosinophil cell count compared to the control group. Eosinophil count decreases cell recruitment and IL-4, IL-5, and IL-13, which affect cell count. Studies on milk-induced leukocytosis and eosinophils verified the plant extract's anti-asthmatic capabilities. In guinea pigs, goats, horses, dogs, and humans, histamine contracts trachea and bronchial muscles. Tracheas in guinea pigs test asthma drugs.Gas chromatography-mass spectrometry(GC-MS),DPPH,ABTS was applied for the identification of chemical constituents in volatile oil from Artemisia argyi and the relative percentage of each component was calculated by area normalization. The study of investigation showed that the ethanolic extract of *Alpinia Calcarata* rhizomes possess 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 The antioxidant and anti- inflam

Keywords Phytochemical, antioxidant, anti-inflammatory, *Alpinia Calcarata* rhizomesmatory.

1. Introduction

Many plant species utilized in herbalism are referred to as "medicinal plants" (also known as "herbology" or "herbal medicine"). It is the application of plants as medicine and the investigation of these applications. The Latin word "herba" and the ancient French word "herbe" are the sources of the English term "herb." These days, a "herb" can be any portion of a plant, including nonwoody plants and fruits, seeds, stems, bark, flowers, leaves, stigmas, and roots. Before, only non-woody plants, such as those derived from trees and shrubs, were referred to as "herbs." These healing plants are also utilized in some types of spiritual activities, as well as for food, flavonoids, medication, and perfume. People used plants for medicinal purposes even before the prehistoric era. Ancient Unani scrolls, Egyptian papyrus, and Chinese literature all contain references to herbal medicines. There is evidence that Unani Hakims, Indian Vaids, and Mediterranean and European cultures have been using plants as medicine for almost 4,000 years. Indigenous cultures in Rome, Egypt, Iran, Africa, and America used herbs in their healing rites. Traditional medicinal systems such as Chinese medicine, Ayurveda, and Unani were developed by other civilizations and involved the methodical use of herbal medicines.[5]

1.1 Inflammatory Cells

• Lymphocytes

An increased understanding of the development and regulation of airway inflammation in asthma followed the discovery and description of subpopulations of lymphocytes, T helper1 cells and T helper2 cells with distinct inflammatory mediator profiles and asthma as a Th2 disease, recognizing the importance of number of families of cytokines and chemokines has advanced our understanding of the development of airway inflammation effects on airway function. After the discovery of these distinct lymphocyte subpopulations in animal models of allergic inflammation, evidence emerged that, in human asthma, a shift, or predilection, toward the Th2-cytokine profile resulted in the eosinophilic inflammation characteristic of asthma. In addition, generation of Th2 cytokines (e.g., interleukin4 (IL-4), IL-5 and IL-3) could also explain the overproduction of IgE, presence of eosinophils, and development of airway hyperresponsiveness. There also may be a reduction in a subgroup of lymphocytes, regulatory T cells, which normally inhibit Th2 cells, as well as an increase in natural killer (NK) cells that release large amounts of Th1 and Th2 cytokines.[7]

1.2 Eosinophils
Increased numbers of eosinophils exist in the airways of most, but not all, persons who have asthma. These cells contain inflammatory enzymes, generate leukotrienes, and express a wide variety of pro-inflammatory cytok

1.3Neutrophils
Neutrophils are increased in the airways and sputum of persons who have severe asthma, during acute exacerbations, and in the presence of smoking. Their pathophysiological role remains uncertain; they may be a determinant of a lack of response to corticosteroid treatment. The regulation of neutrophil recruitment,

1.3 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

1.4 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. [8]

1.5 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

Signs of inflammation
The main signs of inflammation are
• Redness (Latin rubor)
• Heat (calor)
• Swelling (tumor)
• Pain (dolor)

Inflammation may be classified as
A) Acute inflammation
B) Chronic inflammation

A. Acute inflammation
Acute inflammation is immediate and early response to tissue. Main features are listed below
a) Vasodilation
b) Vascular leakage and edema
c) Leukocyte emigration

a) Vasodilation
Brief arteriolar vasoconstriction followed by vasodilation
• Accounts for warmth and redness.
Opens microvascular beds.
• Increased intravascular pressure causes an early transudate into interstitium.

b) Vascular leakage
• Transudate gives way to exudate (proteinrich)
• Increases interstitial osmotic pressure contributing to edema (water and ions)
Five mechanisms known to cause vascular leakiness
1. Histamines, bradykinins, leukotrienes cause an earl, brief (15-30 min) immediate transient response, in the form of endothelial cell contraction that widens intercellular gaps of venules.
2. Cytokines mediators (TNF, IL-1) induce endothelial junction retraction through cytoskeleton reorganization.
3. Severe injuries may cause immediate direct endothelial cell damage (necrosis, detachment) making them leaky until they are repaired (immediate sustained response), or may cause delayed damage as in thermal or UV injury.
4. Marginating and endothelial cell adherent leukocytes may pile-up and damage the endothelium through activation and release of toxic oxygen radicals and proteolytic enzymes.

c) Leucocyte emigration
Leukocytes leave the vasculature routinely through the following sequence of events:
• Margination and rolling
• Adhesion and transmigration
• Chemotaxis and activation Chemical mediators
• Plasmaderived:
➢ Complement, kinins, coagulation factors
➢ Many in "proform" requiring activation (enzymatic cleavage)
• Cellderived:
➢ Preformed, sequestered and released (mast cell histamine)
➢ Synthesized as needed (prostaglandin)

B. Chronic inflammation
Chronic inflammation is defined as prolonged process in which tissue destruction and inflammation occurs at the same time. The characteristic features of chronic inflammation are the presence of chronic inflammatory cells such as lymphocytes, plasma cells and macrophages.[10]

Causes of chronic inflammation
• Chronic inflammation following acute inflammation
• Recurrent attacks of chronic inflammation

Characteristic features
• Necrosis
• Mononuclear cell infiltration
• Proliferative changes
The plant *Alpinia calcarata* has been traditionally used for various diseases. In present study rhizomes of the plant *Alpinia calcarata* have been used which traditionally indicated in the treatment of inflammation

Fig. 1 &2: Leaf and Rhizome of *Alpinia calcarata*

2.0 PLANT DESCRIPTION

Selected plant : *Alpinia calcarata*
2.1 Classification
Kingdom : Plantae
Division : Mangnoliphyta
Class : Liliopsida
Order : Zingiberales
Family : *Zingiberaceae*
Genus : Alpinia
Species : *Alpinia calcarata*
2.2 Botanical description
Alpinia calcarata is a rhizomatous perennial herb with a non-tuberous rootstock, stems slender, about 75 cm tall; leaves simple, alternate, 25 - 32 cm long and 2.5 - 5 cm broad, lanceolate, acuminate, long-pointed, glabrous on both surfaces and shining on the upper surface, scantily hairy along the margin, petioles sheathing; flowers pinkish white, irregular, bisexual, in pendunculate, terminal, dense flowered panicles 8.5 cm long, two flowers together at each node, one

opening earlier than the other, each bearing a pair of bracteoles, the inner one smaller than the outer, bracteoles oblong.[11]

2.3Inflammation may be classified as
a)Acute inflammation
b)Chronic inflammation
2.3 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 [13].
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 [14].
Free radicals are electrically charged molecules, i.e., they have an unpaired electron, which causes them to seek out and capture electrons from other substances in order to neutralize themselves. Although the initial attack causes the free radical to become neutralized, another free radical is formed in the process, causing a chain reaction to occur. And until subsequent free radicals are deactivated

a) 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.

b) 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.42, 43

c) Preliminary phytochemical screening
The ethanolic extract of *Alpinia calcarata* rhizomes were subjected for the following chemical tests for the identification of various active constituents.44, 45

2.4 Detection of carbohydrates: -
• Molish test (General test)
To 2-3 ml of aqueous extract, add few drops of α-naphthol solution in alcohol, shake and add conc. H2SO4 from a test tube. Violet ring is formed at the junction of two liquids. Test for reducing sugar
(a)Fehling's test
Mix 1 ml of fehling's A and 1 ml of fehling's B solution
Boil for 1 min. Add equal volume of test solution. Heat in boiling water bath for5-10 min. First yellow, then brick red precipitate observed.
(b) Benedict's test
Mix equal volume of benedict's reagent and test solution in a test tube. Heat in boiling water bath for 5 min. Solution appears green, yellow or red depending on amount of reducing sugar present in test solution.

2.5 Detection of protein: -
• Biuret test (General test)
3 ml of test solution add 4% NaOH and few drops of 1% CUSO4 solution. Violet or pink color appear.
• Million's test (for proteins)
Mix 2 ml of extract with million's reagent. white precipitate formed. warm precipitate turns brick red or the precipitate dissolves giving red colored solution.

2.6 Detection of proteins and amino acids: -
About 100 mg of extract was dissolved in 10 ml of distilled water and filtered through Whatman No.1 filter paper and the filtrate was subjected to tests for proteins and amino acids.
• Biuret test (General test)
3ml of test solution add 4% NaOH and few drops of 1% CUSO4 solution. Violet or pink color appear.
• Million's test (for proteins)

Mix 2 ml of extract with millions reagent. White precipitate formed. Warm precipitate turns brick red or the precipitate dissolves giving red colored solution
• Ninhydrin test (for amino acid)
About 2 drops of ninhydrin solution were added to 2 ml of test solution. Purple or bluish color appears

2.7 Detection for Fats and oils: -
• Solubility test
Oils are soluble ether, benzene, chloroform but insoluble in 90% ethanol and in water. Hence filter paper get permanently stained with oils

2.8 Detection of steroid: -
• Salkowski reaction
To 2 ml of extract, add 2 ml chloroform and 2 ml con H2SO4 shake well. Chloroform layer appear red and acid layer shows greenish yellow fluorescence.
• Liebermann – burchard reaction
Mix 2 ml extract with chloroform. Add 1-2 ml of acetic anhydride and 2 drops of con H2SO4 from the side of test tube. First red, then blue finally green color appear.

2.9 Detection of glycoside: -
1) Cardiac glycoside
• Legal ‘s test (for cardiac glycoside)
To aqueous or alcoholic extract add 1 ml pyridine and 1 ml sodium nitroprusside. pink to red color appears.
• Test for reducing sugar (for cardiac glycoside)
To 2 ml extract add glacial acetic acid one drop 5% Fecl3 and con H2SO4. Reddish brown color appears at junction of two liquid layer and upper layer appears bluish green.

2) Anthraquinone glycoside
Bontragers test
To 3 ml extract, dil. H2SO4 was added, boiled and filtered. To cold filtrate, equal volume of benzene or chloroform was added and shaken well. The organic solvent was separated and ammonia was added. The ammonical layer turns pink to red.

3) Saponin glycoside
• Foam test
Shake the drug extract or dry powder vigorously with water. Persistant form observed.
• Heamolytic test
Add drug extract or dry powder to one drop of blood placed on glass slide. Heamolytic zone appears

4) Cyanogenic glycoside
Grignard reaction or sodium picrate test
Soak a filter paper strip first in 10% picric acid, Then in 10% sodium carbonate, dry. In conical flask place moistened powdered drug. Cork it, place the above filter paper strip in the slit in cork. Filter paper turn brick red or maroon.

5) Coumarin glycoside
• Coumarin glycoside have aromatic odour
• Alcoholic extract when made alkaline, shows blue or green fluorescence

3.0 Detection for flavonoids
• Shinoda test
To dry powder or extract, add 5ml 95% ethanol, few drops con. Hcl and 0.5 g Magnesium turnings. Pink color observed.
• To small quantity of residue, add lead acetate solution. Yellow color precipitate is formed.
• Addition of increasing amount of NaOH to residue shows yellow coloration, which decolorizes after addition of acid.
3.1 of alkaloid
Aqueous alcoholic and chloroform extract was evaporated separately. To residue dilute Hcl was added. Shaken well and filtered. With the filtrate the following test was performed.
• Dragondorff ‘s test
To 2-3 ml. Filtrate, add few drops of dragondorff‘s reagent. Orange brown precipitate
• Mayers test
To 2-3 ml of filtrate with few drops of mayer’s reagent gives precipitate Hager’s test

2-3 ml filtrate with hager's reagent gives yellow precipitate Wagner's test
2-3 ml of filtrate with wagner's reagent gives reddish brown precipitate *Materials*

***3.2 In vitro* antioxidant activity**

3.3 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.[15]

% Scavenged = $A - A \times A$
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

3.4 Reducing power assay

The reducing power of the extract was determined by the method. 1 ml of the extract solution (25, 50, 100, 200 and 400 µg/ml) was mixed with 2.5 ml phosphate buffer (0.2 M, Ph 6.6) and 2.5 ml of potassium ferricyanide ([K2 Fe (CN)6] (10g/l)), then the mixture was incubated at 500 C for 20 minutes. A portion (2.5ml) of trichloroacetic acid (TCA) (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally, 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water and 0.5ml ferric chloride (FeCl3. 0.1%) and absorbance was measured at 700 nm in UV- visible spectrophotometer. The experiments were performed in triplicate. Increased absorbance of reaction mixture indicates stronger reducing
power.

***3.5 In vitro* anti-inflammatory activity**

3.6 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 720 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.

***3.7 In vitro* anti-inflammatory activity**

3.8 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 720 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.chemical at varying doses for the sample (0µg/ml–2500 µg/ml) and STD (0µg/ml–50 µg/ml) were generated. The plate was left in the dark for thirty minutes. Using a micro plate reader (iMark, BioRad), the decolorization was measured at 495 nm at the conclusion of the incubation. The control was a reaction mixture that contained 20µl of deionized water. The scavenging activity in relation to the control was shown as "% inhibition." Utilizing Software Graph Pad Prism 6, IC-50 was computed.[17]

3.9 ABTS radical scavenging assay -

Experimental Details

To generate the ABTS free radical reagent, APS (2.45 mM) and ABTS (7 mM) solution were mixed and diluted 100 times. This resulted in the synthesis of ABTS radicals. In a 96-well plate, 200µl of ABTS free radical reagent and 10µl of various sample stocks (0–5%) were added to the standard (ascorbic acid, 1 mg/ml) and allowed to sit at room temperature for 10 minutes in the dark. Using an iMark microplate reader (BioRad USA), the absorbance of the decolorization was measured at 750 nm after incubation. Results pertaining to the negative control were displayed. Utilizing Software Graph Pad Prism 6, IC-50 was computed.

4.0 Collection & authentification of *alpinia calcarata* rhizome

The rhizomes of *Alpinia calcarata* were collected and authentified.

4.1 Extraction of plant material

Alpinia calcarata rhizomes were collected, washed and shade dried. Dried rhizomes were crinkled in to powdered form, weighed out. Extraction of coarse powder was done by soxhlet extraction with ethanol. The percentage yield of the product was found to be 17 % w/w.

Fig.3 Extraction of plant material

5.0 Results

5.1 Preliminary phytochemical screening of ethanolic extract of *alpinia calcarata* rhizomes

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. 1 Preliminary phytochemical screening

Sl. No:	**Constituents**	**Presence/absence**
1	Carbohydrate	+
2	Proteins	+
3	Aminoacids	_
4	Fatsandoils	_
5	Steroids	+
6	Cardiacglycosides	+
7	Anthraquinoneglycoside	_
8	Saponinglycosides	_
9	Cyanogenicglycosides	_
10	Coumaringlycosides	+
11	Flavonoids	_
12	Alkaloids	+
13	Tannins	+
14	Phenol	+

(+:presence,-:absence)

Fig 4 & 5 Phytochemical screening of the plant

***5.2 Invitro*antioxidantactivity**

Hydrogenperoxidescavenging

The hydrogen peroxide scavenging activity of ethanolic extract of *Alpinia calcarata*rhizomes was determined. The percentage hydrogen peroxide scavenging ability of the testextract increased in a dose dependent manner and the reference standard, ascorbic acid (100µg/ml) exhibited 60.23% hydrogen peroxide scavenging activity. The maximum hydrogenperoxide scavenging activity shown by ethanolic extract of *Alpinia calcarata* rhizomes wasfoundtobe53.3%at400µg/ml.

TableNo.2:Hydrogenperoxidescavengingactivityofethanolicextractof*Alpiniacalcarata* rhizomes

Slno	Concentration(µg/ml)	Absorbance[A]	%inhibition
1	25	0.632±0.0002	16.16
2	50	0.539±0.0051	28.5
3	100	0.474±0.0054	37.04
4	200	0.414±0.0004	45
5	400	0.357±0.0022	52.3
6	Ascorbicacid(100µg/ml)	0.256±0.046	61.23

(ValuesareMean±S.E.M.,wheren=6)ineachgroup,$P<0.05^{*}$,$P<0.01^{**}$(significant)comparedwithcontrol.Statisticalanalysiswasdonebyone-wayanalysisofvariance(ANOVA)followedbyDunnett'smultiplecomparisontest.

Fig. 6 :Hydrogenperoxidescavengingactivityofethanolicextractof*Alpiniacalcarata*rhizome.

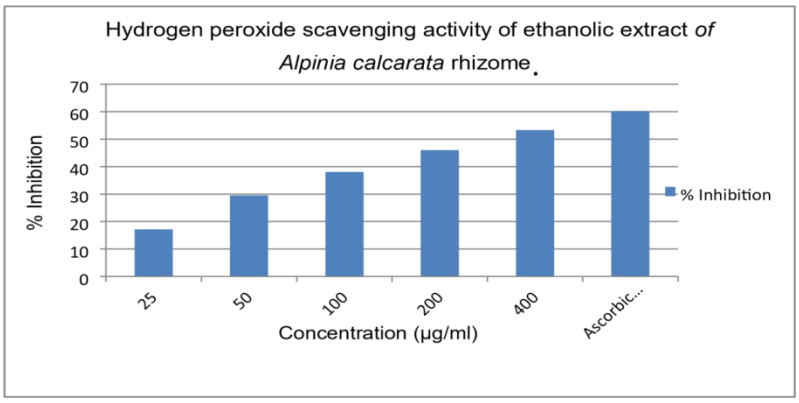


Fig. 6 Hydrogenperoxidescavengingactivity

5.3 Reducingpowerassay

Increase in absorbance of the extract indicates the reducing power of the test sample. ReducingpowerofethanolicextractofAlpiniacalcaratarhizomesincreasedwithincreasingconcentration.Resultsare expressedbelow

TableNo.3:Reducingpoweractivityofethanolicextractof*Alpiniacalcarata* rhizomes

Slno	Concentration(µg/ml)	Absorbance[A]
1	25	0.782±0.32
2	50	0.891±0.21
3	100	1.3±0.35
4	200	1.4±0.42
5	400	1.56±0.82

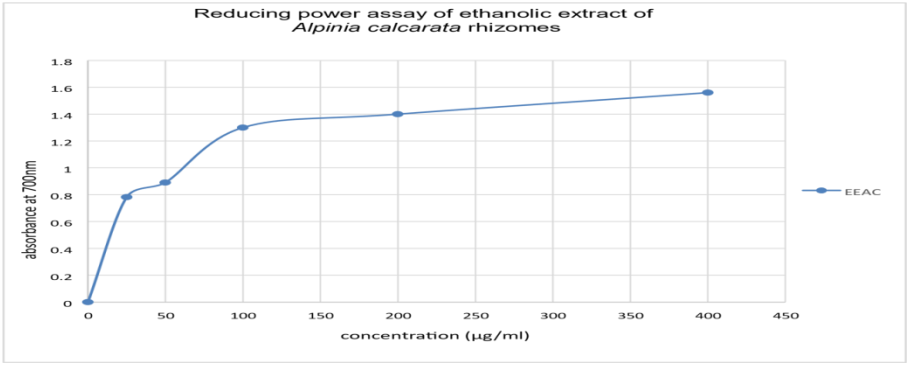


Fig.7 Redussing power assay of ethanoloic extract of Alpinia Calcarata

5.4 Methodfortestingantioxidant activity:

5.5 GasChromatography-MassSpectrometry(GC-MS)analysisofessentialoil:-

Inaseparatingfunnel,10µlofthesample(50mg/ml)wasobtainedandagitatedwith10mlofwaterandethylacetateina1:4ratio(add 2.5µlwaterto7.5µlEthylAcetate).Intherotaryevaporator,theupper layerwascollectedandcondensedto1milliliter.Followingtheaddition of 50 µl of N, O-Bis (trimethylsilyl)trifluoroacetamide and trimethylchlorosilane(BSTFA+TMCS), 10 µl of pyridine was added. Prepare a 100µl solution containing 1µl ofTMCSand99µlofBSTFAforBSTFA+TMCS.ThesampleswereremovedintoGCvialsandnitrogengaswasusedtodrythem.Prio rtoGC-MSanalysis,materialswerefinallydissolvedinmethanol.Theprogrammingoftheacquiredsamplesisoutlinedbelow.

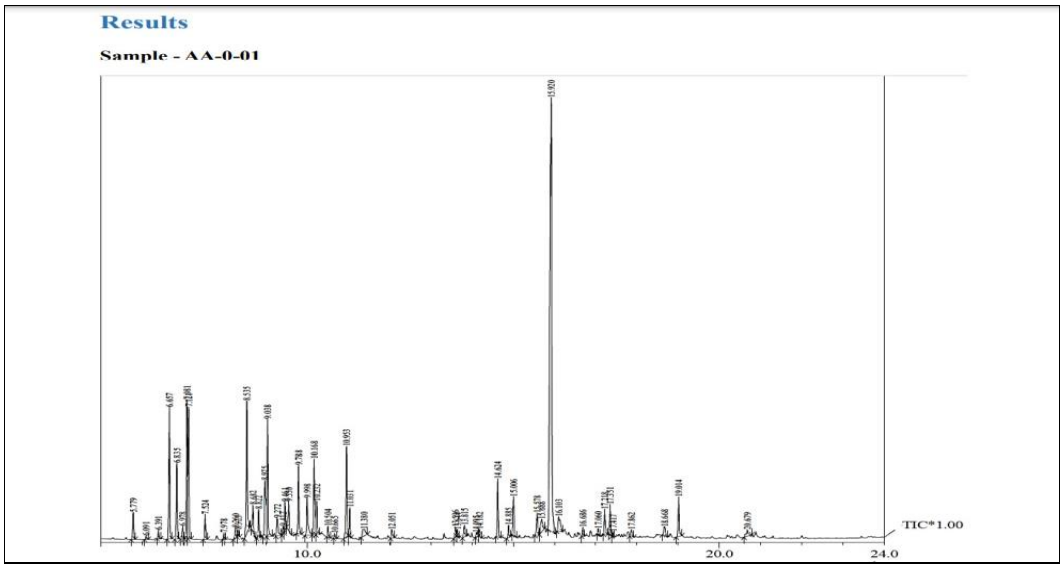


Fig.8 GasChromatography-MassSpectrometry(GC-MS)

5.6 Diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging method :-Reagents6.3mMDPPHinmethanol:
0.1 ml of 0.1 mM DPPH solution was added to 5µl of varied stock of the test substance forSample (0µg/ml-2500 µg/ml) and for STD (0µg/ml-50 µg/ml) on a 96 well plate. Theexperiment was conducted in triplicate, and blanks with 0.2 ml DMSO/Methanol and 5 µlchemicalatvaryingdosesforthesample(0µg/ml–2500µg/ml)andSTD(0µg/ml–50µg/ml)weregenerated.Theplatewasleftinthedarkforthirtyminutes. Usingamicroplatereader(iMark, BioRad), the decolorization was measured at 495 nm at the conclusion of theincubation. The control was a reaction mixture that contained 20µl of deionized water. Thescavengingactivityinrelationtothecontrol wasshownas"%inhibition."UtilizingSoftwareGraphPadPrism6,IC-50wascomputed.

Calculations:-
DPPHScavengingactivity=((Abs$_{Control}$-Abs$_{Sample}$)/Abs$_{Control}$)×100
Table 4:

Samplecode	**IC50value(µg/ml)**
Ascorbicacid	6.097
AA-O-01	298.9

Details of the Experiment To generate the ABTS free radical reagent, APS (2.45 mM) andABTS (7 mM) solution were mixed and diluted 100 times. This resulted in the synthesis ofABTS radicals. In a 96-well plate, 200µl of ABTS free radical reagent and 10µl of varioussample stocks (0–5%) were added to the standard (ascorbic acid, 1 mg/ml) and allowed tosit at room temperature for 10 minutes in the dark. Using an iMark microplate reader(BioRadUSA),theabsorbanceofthedecolorizationwasmeasuredat750nmafterincubation. Results pertaining to the negative control were displayed. Utilizing SoftwareGraphPadPrism6,IC-50was computed.Findings:

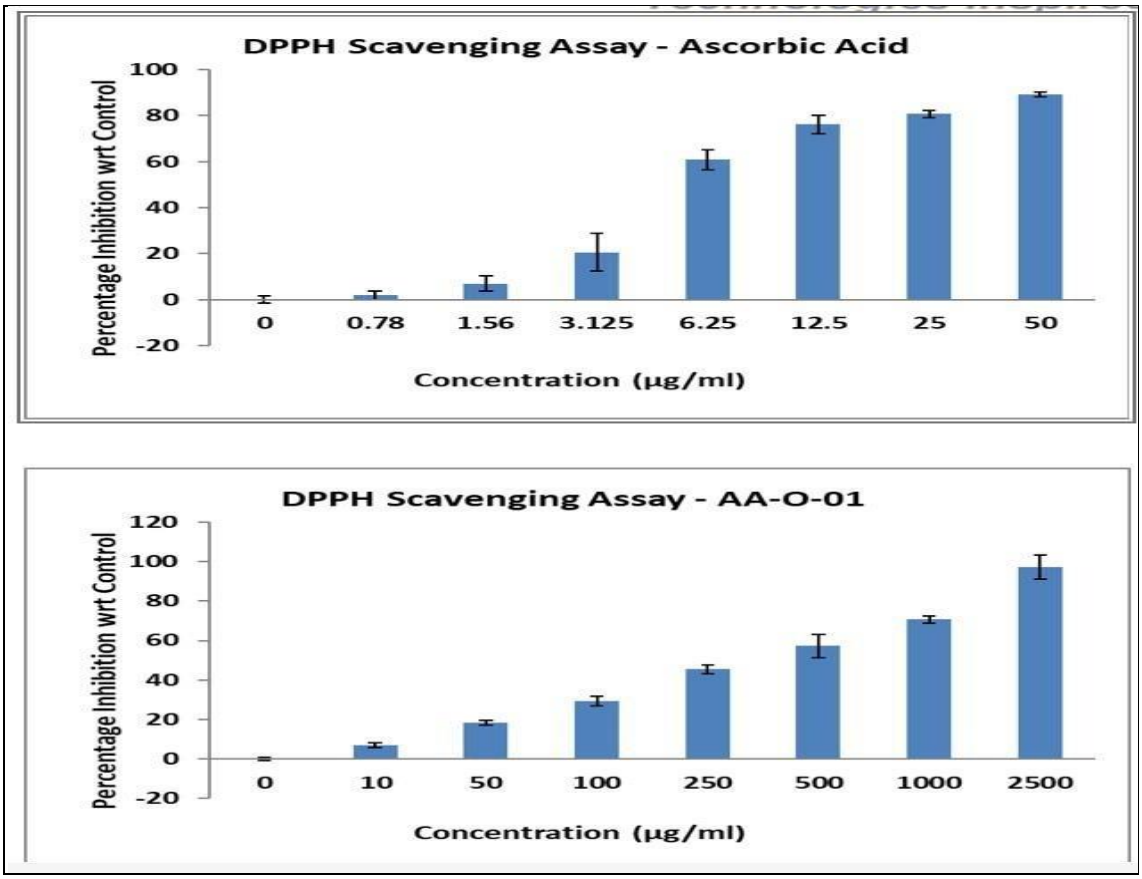


Fig 9& 10 Diphenyl-1- picrylhydrazyl (DPPH) free radical scavenging

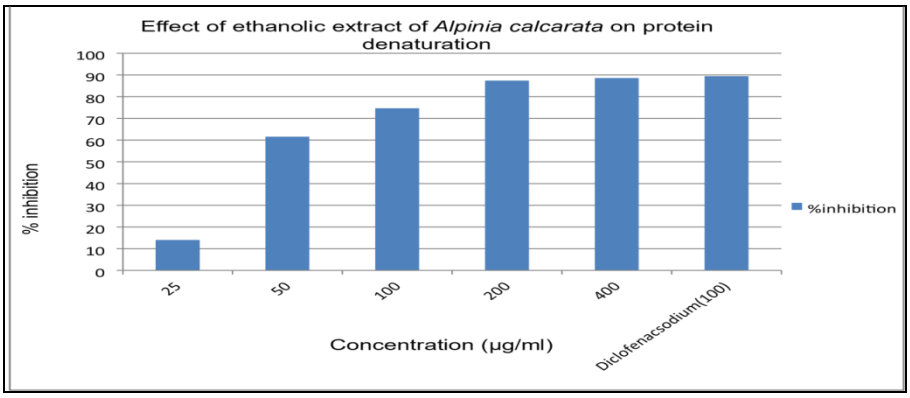
5.7 *Invitro*anti-inflammatoryactivity

Proteindenaturation: As part of the evaluation of anti-inflammatory activity, ability of plant extract on proteindenaturation was studied. It was effective in inhibiting heat induced protein denaturation.Diclofenac sodium a standard anti-inflammatory agent possesses maximum % inhibition. Theethanolicextractoftheplant*Alpiniacalcarata*rhizomepossesssignificant%inhibitionactivityatconcentration200µg/mland 400µg/ml.

TableNo.5:Effectofethanolicextractof*Alpiniacalcarata* onproteindenaturation

Slno	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	Diclofenacsodium(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)comparedwithcontrol.Statisticalanalysiswasdonebyone-wayanalysisofvariance(ANOVA)followedbyDunnett'smultiplecomparisontest.



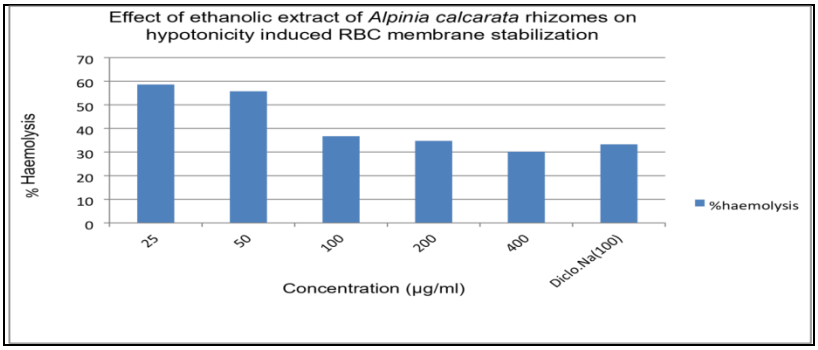
FigureNo11:Effectofethanolicextract*ofAlpiniacalcarata*onproteindenaturationRabbitredbloodcellmembranestabilizationmethod

Theethanolicextractof*Alpiniacalcarata*rhizomehadshownsignificantinhibitionofhaemolysis or the active RBC membrane stabilization comparing to diclofenac sodium thereference standard. The maximum percentage protection shown by test extract was 69.82% at400µg/mlandminimumpercentageprotectionwasfoundtobe41.4at25µg/ml.Thereferencestanddiclofenac sodiumpossesses66.75%atconcentration100µg/ml.

Table No.6: Effect of ethanolic extract of *Alpinia calcarata* rhizomes on hypo tonicity inducedRBCmembranestabilization.

Slno	Concentration(µg/ml)	Absorbance[A]	%Protection	%Haemolysis
1	25	0.61±0.03	41.4	58.6
2	50	0.58±0.002	44.3	55.7
3	100	0.382±0.004	63.3	36.7
4	200	0.36±0.009	65.3	34.7
5	400	0.32±0.007	69.82	30.18
6	Diclofenac sodium (100 µg/ml)	0.34±0.008	66.75	33.25

(ValuesareMean±S.E.M.,wheren=6)ineachgroup,P<0.05*,P<0.01**(significant)comparedwithcontrol.Statisticalanalysiswasdonebyone-wayanalysisofvariance(ANOVA)followedbyDunnett'smultiplecomparisontest.



FigureNo.12:Effectofethanolicextractof*Alpiniacalcarata*rhizomeson hypo tonicityinducedRBCmembranestabilization

DISCUSSION

Alpinia calcarata rhizome ethanolic extract phytochemical composition was examined. Rhizome extract contained phenol, tannins, cardiac glycosides, flavonoids, proteins, and carbohydrates. Plantchemical components synthesize complex compounds and evaluate biological activity. Plants containbioactive phytochemicals. Main and secondary metabolites. Secondary metabolites include alkaloids,terpenoids, and phenolic chemicals; main metabolites are carbohydrates, amino acids, proteins, andchlorophyll. Plant secondary metabolites have medical benefits. Thus, finding new bioactivechemicals requires extensive plant species screening [17, 18]. Phytochemicals like flavonoids areantioxidant secondary metabolites. Their biological mechanisms include heart protection, cell growth,and

anti-aging. Tannins damage plant viruses, bacteria, and fungi. Research reduces their risk ofcoronary heart disease. The study validated the plant's bronchodialotor characteristics, aiding asthmatreatment [19, 20].
An ethanolic Alpinia calcarata rhizome extract reduces histamine-induced tissue contraction.
Histamine (10 g/ml) dosages created a concentration response curve. The study found that Alpiniacalcarata rhizome ethanolic extract at 0.8 mg/ml significantly reduces contracture. The ethanolic. Alpinia calcarata rhizome extract dose-dependently scavenged hydrogen peroxide. H2O2, aphysiologically significant non-radical oxidant, is produced through tissue oxidation. The weakoxidant hydrogen peroxide can directly inactivate certain enzymes by oxidizing crucial thiol (-SH)groups.Asaconclusiontheethanolicextractofthe*Alpiniacalcarata*rhizomesshowedhydrogenperoxide activityascomparedtostandardascorbic acid(100µg/ml).
Protein denaturation is linked to inflammation in numerous studies. Autoantigens may result fromtissue protein denaturation. Anti-inflammatory drugs that prevent protein denaturation may be helpful.Denaturation likely alters electrostatic hydrogen, hydrophobic, and disulfide bonds. Some nonsteroidalanti-inflammatory medicines stabilize heat-treated protein at physiological pH [26, 27].Concentration-dependent protein denaturation was inhibited by Alpinia calcarata rhizome ethanolicextract. The rhizome plant extract showed substantial in vitro anti-inflammatory properties. A plant'sethanolic extract stabilizes membranes by suppressing hypotonicity, boosting anti-inflammatoryeffects. Cell vitality depends on RBC membrane integrity. Hypotonic medium causes hemolysis,membrane lysis, and hemoglobin oxidation. Early inflammation is prevented by membrane-stabilizingdrugs. Zingiberaceae perennial Alpinia calcarata is rhizomatous. Traditional therapies includedasthma, bronchitis, rheumatoid arthritis, stomachic ailment, diabetes, and heart disease [28, 29].Alpinia calcarata rhizome ethanolic extract is phytochemically and pharmacologically studied.Ethanol-soxhlet-extracted powdered rhizomes. In early phytochemical screening, carbohydrates,cardiac glycoside, protein, alkaloids, steroids, flavonoids, tannins, and phenols were found.Ingredients may reflect plant biology. The 2000 mg/kg ethanolic Alpinia calcarata rhizome extractwas not harmful. The most clinically and economically burdensome asthma is allergic andinflammatory. Airway disease asthma. Smooth muscle spasm and histamine production causebronchial asthma blowout constriction. A hydrogen peroxide-scavenging Alpinia calcarata rhizomeethanol extract. Hydrogen peroxide scavenged less than ascorbic acid. Increasing Alpinia calcaratarhizome ethanolic extract dramatically lowered power. In vitro, ethanolic Alpinia calcarata rhizomeextract stabilized rabbit red blood cell membranes and prevented protein denaturation [28-30].Herbal formulations used in the treatment of asthma include some anti-stress herbs toenableadoptiontostresssinceexcessivestressornervousdebility mayaggravatesymptomsofasthma.Thenormalizationeffectofanadaptogencanbeobservedinmilkinducedleukocytosisafter administration of milk. Also eosinophil play a pivotal role in the pathogenesis of allergicdisorders. The plant extract showed marked protection against eosinophil cell count, which is ahallmarkofallergicasthma.

Conclusion

The antioxidantproperty was studiedby hydrogen peroxide scavenging assay andreducing power assay. Hydrogen peroxide scavenging ability of ethanolic extract of *Alpiniacalcarata* rhizomes revealed that the extract scavenge the hydrogen peroxide. However, thehydrogenperoxidescavengingabilitywaslowcomparingtostandard(ascorbicacid).Reducingpowerofethanolicextractof*Alpiniacalcarata*rhizomessignificantlyincreasedwithincreasingconcentration.The*invitro*anti-inflammatorypotentialofethanolicextractof*Alpiniacalcarata*rhizomes had shown concentration dependent inhibition of protein denaturation, hypotonicityinducedhaemolysisofrabbitredbloodcellmembrane stabilization.
Theresultoftheinvestigationshowedthattheethanolicextractof *Alpiniacalcarata*rhizomes possess anti asthmatic activity. The antioxidant and antiinflammatory property of theplantalsosupportsitsanti-asthmaticproperty.Drugseffectiveinasthmaaremostlysteroidalinnature. Phytochemical analysis showed presence of flavonoid and steroids. The anti-asthmaticproperty showed by the plant may be because of these chemical moieties. The results obtainedin the study supports the traditional and also demands further research and to isolate andcharacterizeactive principlesresponsibleforanti-asthmatic activity.

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