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# PHYTOCHEMICAL AND ANTI-OXIDANT ACTIVITY OF ETHANOLIC EXTRACT OF *ALPINIA CALCARATA, ROSC*

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**ABSTRACT**

Natural products of plant origin have played a vital role in the development of new therapeutic agents. The ethanolic extract of the plant *Alpinia calcarata,Rosc.,*(Zingiberaceae) was found to have Anti-oxidant activity . About 12 species of alpinia are found in india. [1] The plant rhizome was used in stomachic, aphrodisiac, tonic, diuretic, expectorant, useful in headache, lumbago, rheumatic pains, sore throat, sore eructations, stuttering, and diseases of kidney. [2] Treatment of Male adult albino rats weighing (150-200 g) with the extract possess strong anti-oxidant activity, evidence by the free radical scavenging property. Further isolation of the bioactive constituent in the plant extract would certainly help to ascertain its potency against various ailments that are induced by oxidative stress. Ethanolic extract of *Alpinia calcarata* also exhibiting significant activity in quenching 1, 1,-di phenyl-2-picryl hydrazyl (DPPH) radicals, there by indicating its potent anti-oxidant effects. [3]

**KEYWORDS:** Alpinia calcarata, Anti-oxidant activity, Phytochemical Studies, Free Radicals.

## INTRODUCTION

Natural products have primarily been isolated from plants used in folklore and traditional medicinal systems of various regions and countries. [4] The plant may be considered a biosynthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by men, but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc, that exerts a physiological effect. [5] The compounds that are responsible for therapeutic effect are usually the secondary metabolites .A systematic study of a crude drug embraces through consideration of both primary and secondary metabolites.

Free radicals are molecules that contain unpaired electrons. The unpaired electron is highly reactive that either burns a molecule (causes oxidative damage) or is passed from molecule to molecule turning the recipient into a free radical and neutralizing the donor. [6] Free radicals attack unsaturated fatty acids in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzyme and receptor activity leading to cell inactivation. Cellular macromolecules are vulnerable to free radical damage: lipids, protein and nucleic acids can all be damaged. Free radical damage to LDL [Low Density Lipids] can lead to atherosclerosis. [7] Free radicals have implicated in cancer, Alzheimer's disease, inflammatory disease, ischemic-reperfusion injury and a myriad of other disease conditions. [8]

For our present study, preliminary phytochemical studies [9] were carried out to characterize the therapeutically active constituents from the plant Alpinia calcarata, Rosc.

## MATERIALS AND METHODS

### Preliminary phytochemical screening

### Collection of plant material

The plant rhizomes Alpinia calcarata, Rosc. were collected from Nagercoil, Tamilnadu. The rhizome were washed in running water , cut into small piece and then shade dried , coarse powdered and used for extraction.

### Purification of solvents

The solvents obtained commercially (LR-Grade) were purified by the fractional distillation.

### Extraction of plant material

The plant material rhizomes were collected, shade dried, coarsely powdered in a blender. The coarse powder was successively extracted in a soxhlet extractor with hexane, chloroform, ethyl acetate, and ethanol by hot percolation for 3 days. (Table 1)

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The solvent was removed by distillation over boiling water bath and remaining under reduced pressure. The residues was stored in the desiccators and used for phytochemical studies. [10]

### *In Vitro* Antioxidant Activity

### Test procedure

#### a. Free Radical Scavenging using 1, 1-Diphenyl - 2 - Picryl Hydrazyl (DPPH) Radical

The hydrogen donating ability of alcoholic extract was examined in the presence of DPPH stable radical. 1 ml of 0.3mM DPPH ethanol solution was added to 2.5ml of sample solution of different concentration and allowed to react at room temperature. After 30min the absorbance values were measured at 517nm Ethanol (1ml) plus plant extract solution was used as a blank. DPPH solution (1ml, 0.3mM) plus ethanol (2.5ml) served as negative control. The positive control was those using the standard (Ascorbic acid) solutions. [11]

#### b. Nitric Oxide Radical Scavenging Assay

Various concentrations of the alcoholic extracts and sodium nitroprusside (10mm) in phosphate buffer saline(0.025m,$P^H$ 7.4) in a final volume of 3ml was incubated at $25^0$ c for 150 min. control experiments without the test compounds but with equivalent amount of buffer were prepared in the same manner as done for the test. Thereafter,oral incubation solution was removed and diluted with 0.5 ml Griess's reagent (1% Sulphanilamide, 2% O-phospharic acid and 0.1% Naphthyethylene diamine dihydrochloride) and allow to reach for 30min.The percentage inhibition was calculated. The experiment was done in triplicate using curcumin (50-800µg/ml) is positive control. [11]

#### c. Deoxyribose Degradation Assay

The decomposing effect of alcoholic extract of alcoholic extract on hydroxyl radicals was determined by the assay of malondialdehyde chromogen formation due to 2-deoxy-2-ribose degradation. The assay mixture contained in a final volume of 1ml.100µl of 28mM 2-deoxy 2-ribose dissolved in phosphate buffer, 200 µl of 200 mM ferric chloride (1:1v/v) and 1.04 mM EDTA and 100 µl of 100 µm ascorbic acid. Percentage inhibition of deoxyribose degradation was calculated Quercetin was used as standard. [11] (Table 2)

### High performance thin layer chromatography

The plant materials under study, each (1gm) were extracted in a soxhlet extractor with hexane, chloroform, ethyl acetate, and ethanol successively on a water bath and filtered and made upto 10 ml in a staneard flask. [12]

### Development of HPTLC finger print

Samples were applied in a concentration 10 µl using camag linomat IV sample applicators aluminium sheets precoated with silica gel merck 60 F 254, 0.2 mm layer thickness (10×10cm) were used as stationary phase (Table 3)

## RESULTS AND DISCUSSION

**Table 1.** Preliminary Phytochemical Test for Different Extracts

| Sr. No. | Test | Hexane | Chloroform | Ethyl acetate | Ethanol |
|---|---|---|---|---|---|
| 1 | Alkaloid | - | - | + | + |
| 2 | Glyciside | - | - | - | - |
| 3 | Anthraquinone | - | - | - | - |
| 4 | Terpenoid | + | + | + | + |
| 5 | Steroid | + | + | + | + |
| 6 | Flavonoids | - | + | + | + |
| 7 | Phenols | - | - | - | + |
| 8 | Tannins | - | - | - | + |
| 9 | Sugers | - | - | + | + |
| 10 | Quinines | - | - | - | - |
| 11 | Saponins | - | - | - | - |
| 12 | Proteins | - | - | + | + |
| 13 | Resin | - | - | - | + |

**Table 2.** Anti-Oxidant Activity of Alcoholic Extract By Different Models

| | 50% inhibitory concentration $IC_{50}$(mg/ml) | | |
|---|---|---|---|
| **Drug** | **DPPH** | **$NO^-$** | **$OH^-$** |
| Extract | 0.085 ± 1.32 | 0.177 ± 1.46 | 0.39 ± 1.53 |
| Ascorbic acid | 0.03 ± 0.06 | | |
| Quercetin | | | 0.112 ± 11.2 |
| curcumin | | 0.076 ± 9.33 | |

Mean ± SEM

**Table 3.** HPTLC Fingerprints of Various Extracts

| Sr. No. | Extracts | Solvent System | No. of Spots | Rf Value | |
|---|---|---|---|---|---|
| | | | | Rf Value | % Peak Areas |
| 1 | Hexane | Hexane: Ethyl Acetate 7 : 3 | 6 | 0.10 | 7.3 |
| | | | | 0.23 | 13.42 |
| | | | | 0.38 | 8.17 |
| | | | | 0.48 | 41.40 |
| | | | | 0.71 | 7.55 |
| | | | | 0.80 | 22.55 |
| 2 | Chloroform | Chloroform : Ethyl Acetate 8 : 2 | 9 | 0.07 | 8.2 |
| | | | | 0.08 | 8.2 |
| | | | | 0.12 | 7.2 |
| | | | | 0.28 | 41 |
| | | | | 0.36 | 14.03 |
| | | | | 0.50 | 40.71 |
| | | | | 0.59 | 8.55 |
| | | | | 0.67 | 6.94 |
| | | | | 0.83 | 1.82 |
| 3 | Ethyl Acetate | Ethyl Acetate : Methanol 9 : 1 | 2 | 0.05 | 8.5 |
| | | | | 0.83 | 91.5 |
| 4 | Ethanol | Chloroform : Methanol 9 : 1 | c2 | 0.08 | 68.7 |
| | | | | 0.86 | 31.3 |
| 5 | Ethanol | Acetonitrile : Water 8.5 : 1.5 | 4 | 0.13 | 26.5 |
| | | | | 0.37 | 24.6 |
| | | | | 0.51 | 23.3 |
| | | | | 0.60 | 25.7 |

## CONCLUSION

The preliminary phytochemical investigation shows the presence of alkaloids, flavonoids, terpenoids, phenols, tannins, proteins and carbohydrates. It also reveals that the various biological activities and therapeutic uses exhibited by the drug plant possess strong anti-oxidant activity, evidence by the free radical scavenging property. Further isolation of bioactive constituent in the plant extract would certainly help to ascertain its potency against various ailments that are induced by oxidative stress. HPTLC finger prints of the n-hexane extract at λ 254 nm shows the presence of 6 spots with Rf value 0.10, 0.23, 0.38, 0.48, 0.71, 0.80. Chloroform extract at λ 254 nm shows the presence of 9 spots with Rf value 0.07, 0.08, 0.12, 0.28, 0.36, 0.50, 0.59, 0.67 and 0.83 respectively. Ethyl acetate extract at λ 254 nm shows the presence of 2 spots with Rf value 0.05 and 0.83 respectively out of 2 spots Rf value 0.83 shows maximum peak area 91.5 which is found to be major and prominent than other spots was due to flavanoid which was confirmed by UV light. HPTLC fingerprint analysis can be used as a diagnostic tool for the correct identification of the plant. The adulterants if any in this plant material can be easily identified by using these results.


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