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Original Article

Comparative analysis of leaf and tuber extracts of *Alpinia Calcarata*

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ABSTRACT

Phytochemical screening of leaf and tuber revealed the presence of essential secondary metabolites. Acetone and methanol extracts of tuber were rich in most of the metabolites like saponins, flavonoid, steroids and tannin whereas leaf extracts showed the presence of tannin, saponins and flavonoids. Chloroform and methanol extracts of tuber showed mild cytotoxic activity using brine shrimp assay. Antioxidant scavenging property was higher in methanol extract of leaf i.e. 80.68% in DPPH free radical scavenging assay. Nitric oxide scavenging activity was maximum in tuber of hexane extract i.e. 52.36%. According to the study cytotoxic activity and free radical scavenging property, methanolic extract of *A. calcarata* leaf was found promising and hence warrants further analysis.

Key words: DPPH (1, 1-diphenyl-2-picryl hydrazyl) Brine shrimp assay, TLC (Thin layer chromatography), Nitric oxide scavenging assay, *Alpinia calcarata*

INTRODUCTION

Traditionally *Alpinia* rhizome is used for the stomach ailments like flatulence, dyspepsia and vomiting. Same has been used for treating high blood pressure and as diuretic [1]. The rhizomes are bitter, acrid, thermo genic, aromatic, nervine tonic, stimulant, revulsive, carminative stomachic, disinfectant, aphrodisiac, expectorant, broncho-dialator, febrifuge, anti-inflammatory and tonic. They are useful in vitiated conditions of vata and kapha, rheumatoid arthritis, inflammations, cough, asthma, bronchitis, dyspepsia, stomachalgia, obesity and intermittent fevers [2].

The oil from the rhizome is carminative and in moderate doses has an anti- spasmodic action on involuntary muscle tissue, inhibiting excessive peristaltic movement of the intestines [3]. It also possesses bactericidal properties. The oil is also used perfumery industries. The rhizomes are used as a condiment in Indonesia. In Kerala, they are used for seasoning fish and in pickling. The rhizomes are reported to be employed for insecticidal purposes as the essential oil contained in them has a high knock- down effect against houseflies [4]. In the present study biologically active rhizome is compared with the leaves of the plant. Usually all the plants in which rhizome is the useful part result in the complete eradication of the medicinal plant, so it was considered worthwhile to explore the medicinal potential of aerial part that is leaf so as to save the plant from reaching the verge of extinction.

MATERIALS AND METHODS

Collection and processing of plant materials: The leaves and tuber of the plant *Alpinia calcarata* were collected from the medicinal germplasm garden of Regional Plant Resource Centre. The two parts were washed properly and dried in shade at room temperature. Then they were powdered using grinder of lexus make. These powered materials were used for further extraction.

Solvent extraction of plant material: Extraction of plant material was done by Soxhlet method with solvents of increasing polarity from a non-polar (hexane) to a more polar solvent (methanol) to ensure that a wide polarity range of compound could be extracted. During extraction the solvent diffuse into the plant material and solubilise compounds with similar polarity. About 7 gm. of powdered sample of *A. calcarata* leaf and 18 gm. of *A. calcarata* tuber powder was taken in thimbles for extraction. Successive extraction was conducted according to the increasing polarity starting from

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hexane, chloroform, acetone and methanol. The extracts were then concentrated under vacuum in Rota Vapour (Buchii) and semi solid extracts were stored in screw cap vials till further use.

Phytochemical tests: Phytochemical tests for secondary metabolites, starch and carbohydrates were conducted as per the standard protocols[5]. shows the presence of iodine.

Cytotoxic activity of plant extracts: The cytotoxic activity of leaf and tuber extracts *Alpinia calacarata* was done by brine shrimp motility assay [6]. A salt solution was prepared for hatching of brine shrimp larvae. 3.6 gm of potassium chloride (KCl) was dissolved in 200ml of distilled water and brine shrimp eggs were incubated for 24 hrs at 28 ± 2ºC. Different stock solutions of plant extracts were prepared. The cytotoxic activity assay was done in triplicates by taking control, positive control and experimental groups. Control test tubes contained only 20 brine shrimps, positive control contained larva plus ethanol (Vehicle) at different doses, while experimental test tubes contained extracts at different doses. The brine shrimp motility activity was viewed in each hour interval up to 4 hrs. After 24 hours all the samples were tested for live and dead parasites. Percentage inhibition was calculated by comparing experimental samples with the controlled samples.

Antioxidant activity: Antioxidant activity of all the extract was conducted using qualitative and quantitative antioxidant assays.

***Qualitative antioxidant assay*:** Thin layered chromatography based DPPH assay was performed for qualitative analysis.

A stock solution of 2 mg plant extracts in 500 µl of each solvent extract was prepared. The precoated TLC plates 60 F_{254} (Merck Company) were activated at 100^0C for 10 min. The samples were then spotted with the help of micro tips leaving 2 cm from the bottom of the sheet.

Three different types of solvents were prepared,

(I) Benzene: ethanol: ammonium hydroxide (BEA) (intermediate polarity/basic)in the ratio of 45:5:0.5,
(II) Chloroform: Ethyl acetate: Formic acid (CEF) (polar/acidic)(5:4:1) and
(III) Ethyl acetate: methanol: water (EMW) (polar neutral) (40:5.4:4).

0.2 % of DPPH solution was prepared in methanol. After drying of sheets DPPH solution was spread and the resulting bands were observed and Retardation factor (R_f) was calculated. Yellow bands in purple background represent the antioxidant bands of the extracts.

Retardation factor = Distance travelled by the compounds/ Total distance travelled by the solvents. Protocol of Masoko and Eloff [7] was followed for TLC based DPPH assay.

Quantitative antioxidant assays

DPPH radical scavenging assay

The radical scavenging activity of different extracts against DPPH was determined spectrophotometrically by the method of Brand Williams *et al*, [8]. DPPH reacts with an antioxidant compound that can donate hydrogen and it gets reduced. The change in colour (from deep violet to light yellow) was measured. DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The intensity of the yellow colour depends on the amount and nature of radical scavenger present. A reaction mixture containing 500µl of 1 mM DPPH, various concentrations of plant extracts (7.81, 15.62, 31.25, 62.5, 125, 250 and 500 µg/ml) were prepared in methanol. A test tube containing only methanol and 500 µl of DPPH solution was taken as control. Then the tubes were incubated in dark for 30 min at room temperature. The yellow colour chromophore was measured at 517nm. Ascorbic acid was used as standard. The percentage scavenging of DPPH free radical was calculated by following formula.

% scavenged DPPH radical = A control – A sample/A control X 100; Where A control is absorbance of control and A sample is absorbance of sample

Nitric Oxide Scavenging Activity: Nitric oxide scavenging activity was measured by slightly modified methods of Green et al[9]. Nitric oxide radicals (NO) were generated from sodium nitroprusside.1ml of sodium nitroprusside (10mM), 1.5 ml of phosphate buffer saline (0.2M, pH7.4) was added to the different concentrations (12.5,25, 50,100,200 and 400µg/ml) of the plant extracts and incubated for 150 min at 25^0C. After incubation, 1ml of the reaction mixture was treated with 1ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthalene diamine dihydrochloride).The absorbance of the chromatophore was measured at 546nm. Quercetin was used as a standard. The nitric oxide scavenging activity (%) was calculated by the following equation.

Nitric oxide scavenging activity (%) = (A control − A sample) / A control] × 100

Where, 'A- control' is the absorbance of the control reaction (containing all reagents except the test compound), 'A- sample' is the absorbance of the test.

RESULTS AND DISCUSSIONS

Phytochemical Tests: As can be seen from the Table 1, Hexane and chloroform extracts of both tuber and leaf showed the presence of alkaloids. Saponins and flavonoids were present in polar extracts that is acetone and methanol extract of both leaf and tubers. Overall it can be observed that as far as phytochemical tests are concerned leaf and tubers were almost similar except for the presence of tannin in leaf extract and nutritional factor carbohydrate which was found only in tubers. Besides study is in confirmation with previous study where tannin, flavonoids and alkaloids have been reported from the rhizome oil of the same species[11]

Cytotoxic Activity (Bioassay): As can be observed from the comparative figures of four extracts(Fig 1), there was a dose dependent response in most of the extracts. However, Tuber extracts showed higher activity as compared to leaf extracts but that was not statistically significant. Highest activity obtained was 63 % in the chloroform extract of tuber at a dose of 200microgam/ml followed by 61% activity in the methanol extract of tuber at the similar dose. Thus overall it can be said that tubers are medicinally more active in comparison to the leaf extract but the difference of activities is not very significant. Although cytotoxic activity has not been reported from this species but a number of species like A.galanga and A.mutica rhizomes have also shown mild cytotoxic activity against cell lines[12].

Antioxidant activity

Qualitative antioxidant assay: Qualitative antioxidant assay was conducted in three solvent systems BEA, EMW and CEF. Bands were seen in all the three solvents(Table 2), a good separation was seen in the BEA solvent of acetone extracts 9 bands were seen whereas least separation in CEF 1 band was seen of *Alpinia calcarata* leaf and 5 bands were seen of *Alpinia calcarata* tuber. Hexane and chloroform extract of *Alpinia calcarata* leaf gave minimum separation in compare to acetone 8 bands were seen in the BEA solvent of *Alpinia calcarata* leaf whereas 4 bands were seen in hexane extract of tuber and 5 band of chloroform extract of tuber. In methanol extracts least separation 4 bands were seen in *Alpinia calcarata* leaf BEA solvent but no separation was seen in EMW whereas only 2 bands were seen in EMW solvent of *Alpinia calcarata* tuber and no band was seen in BEA and CEF. Streak was seen in many solvent which indicate that antioxidant molecules were in large numbers and very closely situated so that there was no clear band formation. As per the results of qualitative assay it was clear that both leaf and tubers were rich in the number of antioxidant bands corresponding to antioxidant activity.

DPPH radical scavenging assay: *Alpinia calcarata* Leaf and Tuber of hexane, chloroform, acetone and methanol extracts were compared for their DPPH free radical scavenging activity by UV/VIS Spectrophotometer and ascorbic acid was taken as standard. The IC_{50}value of Hexane, Chloroform, Acetone and Methanol were determined. The activity of methanol leaf (80.68%) was higher than that of Tuber (78%) at highest concentration of 250µg/ml (Fig 1). The acetone extract also showed good scavenging activity in both leaf and tuber extracts i.e. 57.86% and 66.61% respectively at highest concentration of 1mg/ml, but hexane and chloroform extracts of both leaf and tuber showed insignificant scavenging activity. Methanol extract of leaf showed significant DPPH free radical activity. The IC_{50} value for methanol leaf and tuber extract was obtained in between 62.5-125µg/ml, , IC_{50} value for Acetone leaf extract was obtained in between 0.5-1mg/ml and the IC_{50} value for Acetone Tuber extract was obtained in between 31.25-62.5 µg/ml. IC_{50} value for ascorbic acid was obtained in between 15.62-31.25µg/ml. Although none of the extract was active as standard but as extract is always a mixture of many molecules which can act antagonistically.

Nitric oxide scavenging assay:- A *calcarata* Leaf and Tuber of hexane, chloroform, acetone and methanol extracts were compared for their nitric oxide scavenging activity by UV/VIS Spectrophotometer and Quercetin was taken as standard. The IC_{50}value of Hexane, Chloroform, Acetone and Methanol were determined. The nitric oxide scavenging activity was found significant only in tuber extracts (Fig 2). The activity of Hexane tuber (% of inhibition 52.36) was higher than that of leaf (% of inhibition 9.90) but Acetone and Methanol extract of leaf showed less scavenging activity when compared to Hexane and chloroform extract. The IC_{50} value for hexane tuber extract was obtained in between 25-50µg/ml, the IC_{50} value for chloroform extracts was obtained in between 50-100µg/ml, IC_{50} value for Quercetin was obtained in between 100-200µg/ml. The study is in confirmation with the study in the rhizome of *Alpinia japonica* where a number of molecules have been isolated and they all showed mild to active Nitric oxide radical scavenging activity [13].

CONCLUSION

Study has thus revealed that there is marginal difference in case of leaf and tuber samples. Both the parts revealed a number of important class of compounds like alkaloids, flavonoids and

sapponins. Tuber extract showed good cytotoxic activity in its hexane extract whereas, antioxidant activity was good in hexane extract of leaf and chloroform extract of tubers. Thus, this study has revealed that along with tuber, leaves are also potential candidate for extensive research work.

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Table 1. Phytochemical elements of leaf and tuber extracts

Phytochemical moiety	Hexane		Chloroform		Acetone		Methanol	
	Tuber	Leaf	Tuber	Leaf	Tuber	Leaf	Tuber	Leaf
Alkaloid	+	+	+	+	-	-	-	-
Saponin	-	-	-	-	+	+	+	+
Flavonoid	-	-	-	-	+	+	+	+
Carbohydrates	-	-	+	-	+	-	+	-
Steroids	-	-	-	-	+	-	+	-
Tannin	-	-	-	-	-	+	-	-
Protein	-	-	-	-	-	-	-	-
Starch	-	-	-	-	-	-	-	-

Table 2:- Qualitative antioxidant assay of *A. calcarata* leaf and tuber

Solvent extracts	R_f value of A. calcarata					
	Leaf			Tuber		
	BEA	EMW	CEF	BEA	EMW	CEF
HEXANE	0.98,0.91,0.83, 0.320.56,0.67 0.19,0.14	Streak	Streak	0.71,0.28, 0.85,0.91	Streak	Streak
CHLOROFORM	0.98,0.91,0.32, 0.240.54,0.20 0.16,0.22	0.88,0.24	0.83,0.56, 0.14,0.08	0.71,0.54, 0.62, 0.84, 0.91	Streak	0.8
ACETONE	0.22,0.98,0.91, 0.15,0.32,0.09, 0.17,0.16,0.09	Streak	0.84	0.25,0.52, 0.61	0.95	1.04,0.61, 0.7, 0.9
METHANOL	0.06,0.11,0.19, 0.22	Streak	Streak	Nil	0.65,0.9	Nil

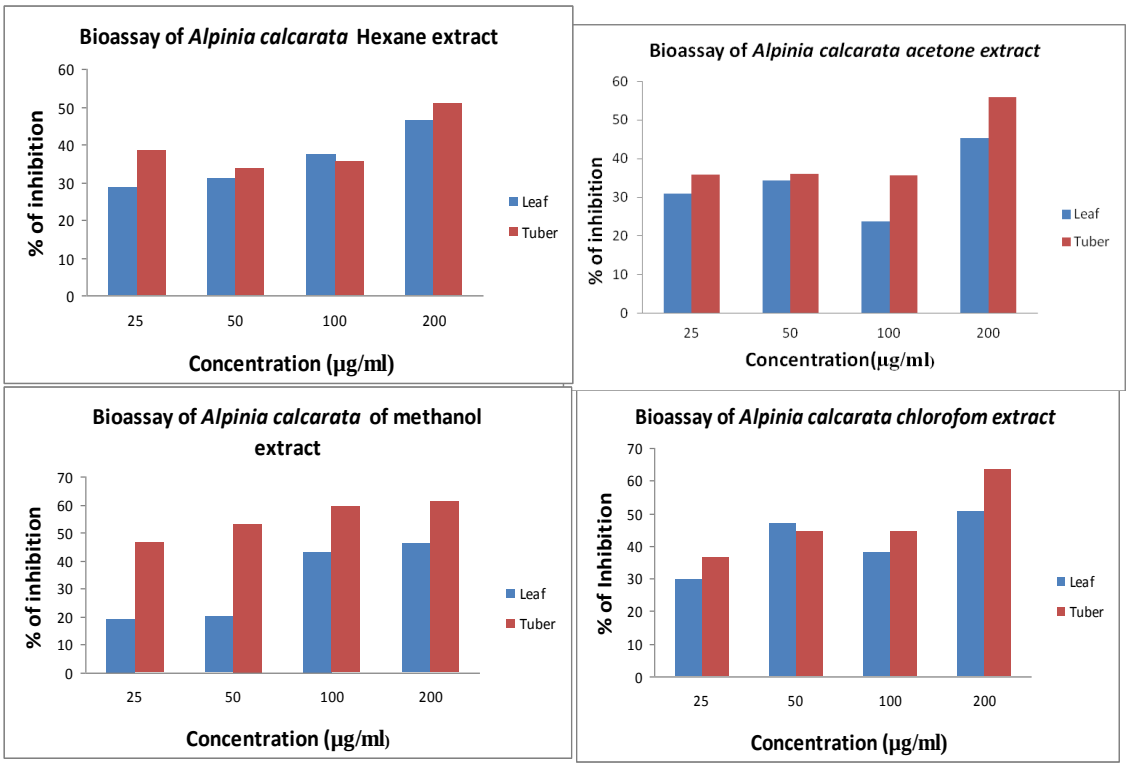

Figure 1. Cytotoxic activity of leaf and rhizome extracts of *Alpinia calcarata*

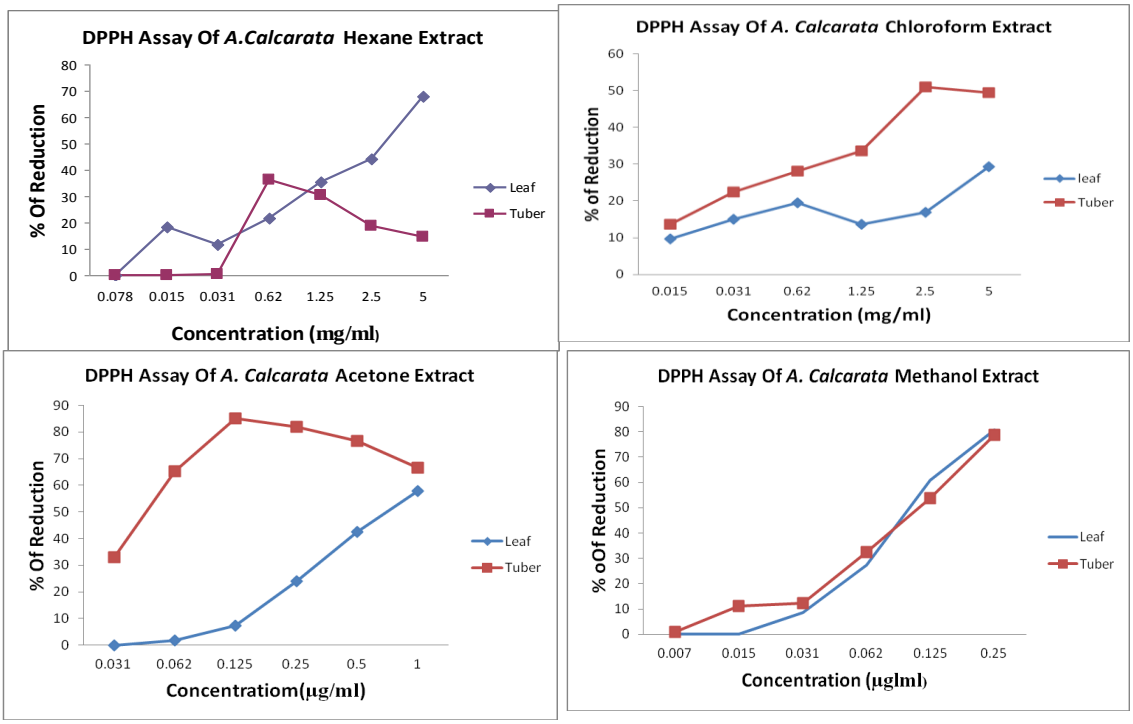

Figure 2. DPPH radical scavenging assay of leaf and tuber extract

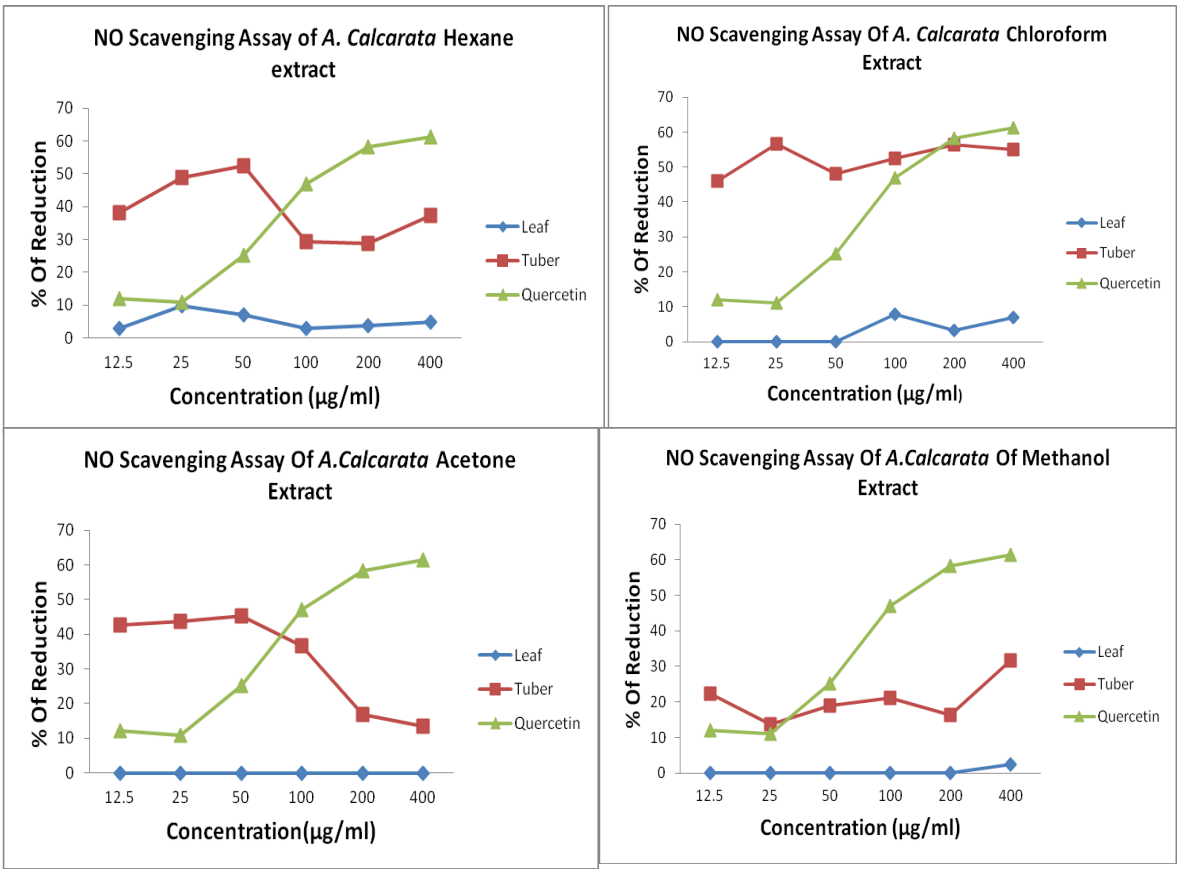

Figure 3: Nitric oxide scavenging assay of leaf and tuber extracts

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