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Original Article

COMPARATIVE STUDY OF ANTIOXIDANT AND ANTICANCER ACTIVITY OF *ALPINIA CALCARATA AND ALPINIA GALANGA*

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ABSTRACT

Objective: The present study was to determine *in vitro* antioxidant and anticancer activity of *Alpinia calcarata* and *Alpinia galanga*.

Methods: The phytochemical screening of rhizome of aqueous extract of *Alpinia calcarata* and *Alpinia galanga* was performed using standard procedures. The total phenolic and flavonoid content were determined by Folin-Ciocalteau and Aluminium chloride reagents. The various antioxidant assays and cytotoxic assays (MTT) for *Alpinia calcarata* and *Alpinia galanga* was performed using standard methods (DPPH radical scavenging assay, Nitric oxide radical scavenging assay, Reducing power assay, Phosphomolybdenum reduction assay).

Results: The preliminary phytochemical screening of *Alpinia calcarata* and *Alpinia galanga* showed the presence of flavonoids, phenols, terpenoids, carbohydrates and proteins. The phenolic content of aqueous extracts of rhizomes of *Alpinia calcarata* was 454.05 µg/mg and *Alpinia galanga* was 480.13 µg/mg and was expressed as gallic acid equivalent. The flavonoid content of aqueous extracts of rhizomes of *Alpinia calcarata* was 36.34 µg/mg and *Alpinia galanga* was 67.68 µg/mg and was expressed as quercetin equivalent. In DPPH assay, *Alpinia galanga* showed 95.36% whereas *Alpinia calcarata* showed 54.54% at 120 µg/ml. The maximum $NO^{\bullet}$ radical scavenging activity was 59.44% for *Alpinia calcarata* and was 73.10% for *Alpinia galanga* at 120 µg/ml concentration. The maximum reducing property was found at the 120 µg/ml of aqueous extract of rhizomes of *Alpinia galanga* which was higher than the *Alpinia calcarata*. In Phosphomolybdenum assay, the aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were 55.47% and 78.38% respectively. The results of the present investigation indicated that rhizome of aqueous extract of *Alpinia galanga* showed the highest antioxidant activity in all the assays than *Alpinia galanga.* The cytotoxicity assay results indicated that rhizome of aqueous extract of *Alpinia galanga* showed 88.36% cell viability whereas *Alpinia calcarata* showed 73.59% cell viability.

Conclusion: The results obtained in the present study indicate that rhizome of *Alpinia galanga* are abundant in phenols and flavanoids which may be useful for the development of the anticancer drug.

Keywords: *Alpinia galanga*, *Alpinia calcarata*, Antioxidant activity, Anticancer activity

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INTRODUCTION

India has a great wealth of medicinal and aromatic plants due to its rich plant diversity. Further, India's unique biogeographical position makes possible the existence of all known types of ecosystems. All the three levels of biodiversity, viz., species diversity, genetic diversity and habitat diversity are found in India. The Indian subcontinent has versatile plant diversity due to its wide-ranging topography and altitudinal differences from sea level to the highest mountain ranges, the immense coastal line in peninsular India, desert in the west, and coolest desert in the eastern regions [1]. The traditional systems of medicine, namely Ayurveda, Siddha and Unani for various herbal preparations use only 2,000 plants out of the total 17,000 flowering plants present. Around 4,500 to 5,000 plant species are utilized by the Indian traditional village physicians, whereas; the oral tradition of the villagers utilizes 5,000 plants for medicinal purposes. In the last decade, for treating various health ailments, the tribe and other traditional communities in India use over 8,000 wild plant species [2].

As 80% of the population in the world for their primary healthcare still depends on traditional medicines, it clearly denotes the important role played by the traditional medicine in treating various infectious diseases [3]. Around 65% of the population in rural India for the primary healthcare purpose uses traditional drugs [4]. Researchers reported that for effective conservation of medicinal plants, first and the foremost important condition are to know the status of existing medicinal plant resources [5].

Alpinia calcarata Roscoe (Family: Zingiberaceae), is a rhizomatous herb, which is commonly used in the traditional medicinal systems in Sri Lanka. *Alpinia calcarata* rhizomes have a broad spectrum of medicinal properties. The rhizomes of *Alpinia calcarata* are known to possess antibacterial, anthelmintic, antifungal [6], antioxidant [7]. Aphrodisiac [8], gastroprotective [9], antidiabetic and anticancer activity. Researchers reported that the rhizomes of *Alpinia calcarata* used to treat high blood pressure, Diuretic, stomach problems, analgesic [10], anticandidal, antiplatelet, antispasmodic [11] antiulcerous hypotensive [12] insecticidal, muscle relaxant and uterine stimulant [13].

Alpinia galanga (L.) (Family: Zingiberaceae is a rhizomatous plant widely distributed in tropical areas and used as a medicine in many countries. The rhizomes of *Alpinia galanga* is widely used to treat rheumatism, bronchitis, diabetes mellitus and loss of appetite. The various bioactive compounds such as Diarabinoside [14], β=steroldiglucosyl caprate [15], Galangoflavonoside [16] and 1-Acetoxychavicol acetate [17] have been isolated. The various parts of the plant also show numerous medicinal properties. Leaf extract was shown to exhibit antibacterial activity and wound healing property [18]. The Seeds of *Alpinia galanga* was found to possess antibacterial activity [19].

The aim of the present investigation was to determine the antioxidant and anticancer activity of rhizome extract of *Alpinia calcarata* and *Alpinia galangal.*

MATERIALS AND METHODS

Collection and authentication of plant materials

The dried rhizomes of *Alpinia calcarata* and *Alpinia galanga* were collected from the market at Mylapore, Chennai (Voucher No. A1002

and A1003) and deposited in Department of Biotechnology, Dr. MGR University, and the rhizomes of plant samples were authenticated by Prof. N. Raaman, Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai, India

Processing of plant material and preparation of crude extract

The dried rhizomes of two different medicinal plants were collected, washed under running tap water followed by distilled water and shade dried for 5 d. The dried rhizomes were ground to fine powder and sieved. Approximately 50 g of finely powdered rhizomes were soaked in 150 ml of distilled water at room temperature for 24 h. The crude extract was filtered using Whatmann No.1 filter paper and then concentrated in vacuum at 40 °C-50 °C (overnight) using a rotary evaporator. The residue was weighed and used for further studies.

Preliminary phytochemical screening

The rhizomes of aqueous extract *Alpinia calcarata* and *Alpinia galanga* was subjected to the qualitative phytochemical screening for the presence of phytoconstituents. Phytochemical test was carried out using standard procedures [21-22].

Determination of total phenolics and total flavonoids content

The total phenolics content was determined according to the Folin-Ciocalteu method [22] and the results were expressed as mg gallic acid equivalent/g of dry weight of extract. While the total flavonoids content of the aqueous extract were measured by the method of [23] and the results expressed as mg quercetin equivalent/g dry weight. All tests were performed in triplicate and mean was centered.

Antioxidant activity

DPPH radical scavenging activity

The antioxidant activity of aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga* (20-120 µg/ml) were evaluated through a free radical scavenging effect on 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. The determination was based on the modified method of [24]. 1 ml of 0.1 mmol DPPH methanolic solution was added into 1 ml of sample extracts. The mixture was thoroughly mixed and kept in the dark for 30 min. The control was prepared by mixing 1 ml of DPPH and 1 ml methanol. The absorbance was measured at 517 nm using a spectrophotometer. Percentage of DPPH radical scavenging activity was calculated as follows.

$$\text{DPPH radical scavenging effect (\%)} = (1 - A_s/A_c) \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample extract or standard

Nitric oxide radical scavenging activity

Nitric oxide radical scavenging capacity of aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga* was measured according to the method described by [25]. 0.1 ml of sodium nitroprusside (10 mmol) in phosphate buffer (0.2 M, pH 7.8) was mixed with a different concentration of aqueous extract (20–120 µg/ml) and incubated at room temperature for 2 h. After the incubation period, 0.2 ml of Griess reagent (2% Phosphoric acid, 0.1% N-(1-Naphthyl) ethylenediamine dihydrochloride and 1% Sulfanilamide,) was added. The absorbance was read at 546 nm against blank. All readings were taken in triplicate and Vitamin C was used as the standard. The % inhibition of niric oxide scavenging of extract and standard was calculated by the following equation.

$$\text{Nitric oxide scavenging effect (\%)} = (1 - A_s/A_c) \times 100$$

Where A_C is the absorbance of the control and A_S is the absorbance in the presence of the sample extract or standard.

Reducing capacity assessment

The reducing power activity was determined by the spectro-photometric method of [26]. An amount of 20–120 µg/ml of aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga* thoroughly mixed with 2.5 ml of 200 mmol phosphate buffer (pH 6.6) followed by the addition of 2.5 ml of 30 mmol potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. To the mixture, 2.5 ml of trichloroacetic acid (600 mmol) was added and centrifuged at 3000 rpm for 10 min. 2.5 ml of supernatant solution was mixed thoroughly with 2.5 ml of distilled water and $FeCl_3$ (0.5 ml, 6 mmol). The antioxidants present in the extract results in the reduction of Fe^{3+} to Fe^{2+}. The Perls Prussian blue colour was observed and read at 700 nm. Vitamin C was used as a positive control.

Phosphomolybdenum reduction assay

The antioxidant capacity of the aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were assessed as described by [27]. The different concentration of aqueous extracts (20-120 µg/ml) was mixed with the reagent solution containing ammonium molybdate (4 mmol), sodium phosphate (28 mmol) and sulphuric acid (600 mmol). The reaction mixture was incubated in a water bath 90 °C for 90 min. The absorbance of the coloured complex was measured at 695 nm. The appropriate solutions of ascorbic acid have been used as a standard reference. Increased the absorbance of the reaction mixture indicates the increase in phosphomolybdenum reduction.

Anticancer activity

Cell line and culture

Human liver cancer HepG2 cell line was obtained from National centre for cell sciences Pune (NCCS). The cells were maintained in Minimal Essential Media supplemented with 10% FBS, Penicillin (100 U/ml), and Streptomycin (100 µg/ml) in a humidified atmosphere of 50 µg/ml CO_2 at 37 °C.

Chemicals

Minimal essential medium **(**MEM) was purchased from Hi-Media Laboratories Fetal bovine serum (FBS) was purchased from Cistron laboratories Trypsin, methyl thiazolyl diphenyl-tetrazolium bromide (MTT), and Dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich, Mumbai.

***In vitro* assay for cytotoxicity activity (MTT assay)**

The Cytotoxicity of extracts on HepG2 cells was determined by the MTT assay [28]. Cells (1×10^5/well) were plated in 1 ml of medium/well in 24-well plates. After 48 h incubation, the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the samples in 0.1% DMSO for 48h at 37 °C. After removal of the sample solution and washing with phosphate-buffered saline (pH 7.4), 200 µl/well (5 mg/ml) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl--tetrazolium bromide cells (MTT) phosphate-buffered saline solution was added. After 4h incubation, 0.04M HCl/isopropanol was added. Viable cells were determined by the absorbance at 570 nm. Measurements were performed and the concentration required for a 50% inhibition of viability (IC50) was determined graphically. The absorbance at 570 nm was measured with a UV-Spectrophotometer using wells without sample containing cells as blanks. The effect of the extracts on the proliferation of liver cancer cells was expressed as the % cell viability, using the following formula:

% cell viability = A570 of treated cells/A570 of control cells × 100%

RESULTS AND DISCUSSION

Phytochemical analysis of aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

The preliminary phytochemical screening of rhizomes of aqueous extracts was done to assess the presence bioactive components. The secondary metabolites are commonly called as phytochemicals that possess strong antioxidant activity and might play an important role in preventing colossal oxidative damage. The secondary metabolites are usually classified through their biosynthetic pathways and are divided into three large families: alkaloids, terpenes and steroids, and phenolic compounds [29]. They are mainly found in storage tissues rather than in vegetative ones, due to their high biological potency [30]. The results of aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were showed the presence of terpenoids, phenols, and flavonoids as shown in table 1. The present study was correlated with the previous report, the ethanolic extract of *Alpinia calcarata* and

Alpinia speciosa showed the presence of alkaloids, flavonoids, phenols, steroids, tannins, proteins and carbohydrates. It was investigated that flavonoids present in *Alpinia calcarata* and *Alpinia speciosa* rhizomes have excellent antioxidant activities and are important bioactive components in rhizomes which can cause inhibition of the oxidative modification of the human lipoprotein [31].

Table 1: Preliminary phytochemical test of rhizome of aqueous extracts of *Alpinia calcarata* and *Alpinia galanga*

S. No.	Test	*Alpinia calcarata*	*Alpinia galanga*
1	Carbohydrates	+	+
2	Proteins	+	+
3	Aminoacids	+	+
4	Alkaloids	-	-
5	Flavanoids	+	+
6	Steriods	+	-
7	Tannins	+	-
8	Terpenoids	+	+
9	Saponins	-	-
10	Phenols	+	+
11	Glycosides	-	-

+Indicates Positive Result; **--**Indicates Negative Result

Total phenol and flavonoid content of aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

Phenolics are the most widespread secondary metabolite in the plant kingdom. These diverse groups of compounds have received much attention as a potential natural antioxidant in terms of their ability to act as both efficient radical scavengers and a metal chelator. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers [32]. The various studies prove that dietary phenolic compounds have a great role in preventing neurodegenerative disorders [33]. Therefore, in the present study, total phenolic content present in the extract was estimated using modified Folin-ciocalteau method and flavonoid content was estimated by $AlCl_3$ method. The phenolic content of aqueous extracts of rhizomes of *Alpinia calcarata* was 454.05 µg/mg and *Alpinia galanga* was 480.13 µg/mg and was expressed as gallic acid equivalent. The flavonoid content of aqueous extracts of rhizomes of *Alpinia calcarata* was 36.34 µg/mg and *Alpinia galanga* was 67.68 µg/mg and was expressed as quercetin equivalent (table 2).

Table 2: Total phenol and flavonoid content of aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

Medicinal plants	Total phenolic content (GAE) µg/mg	Total Flavanoid content (QE) µg/mg
Alpinia calcarata	454.05	36.34
Alpinia galanga	480.13	67.68

GAE-gallic acid equivalent, QE-quercetin equivalent

DPPH radical scavenging assay

In this present study, the antioxidant activity of aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were investigated by using DPPH radical scavenging assay. The DPPH antioxidant assay is based on the ability of DPPH, a stable free radical, to decolourize in the presence of antioxidants. DPPH has the advantage of being unaffected by certain side reactions, such as metal ion chelation and enzyme inhibition brought by various additives. A freshly prepared DPPH solution exhibits a deep purple colour generally disappears when antioxidants present in the medium. Thus the antioxidants present in the extract can quench DPPH free radicals by donating hydrogen atom or by electron transfer and convert them to the colourless product (2,2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine, resulting in a decreasing absorbance at the 517 nm [34]. The mixture of 1 ml methanol and 1 ml of DPPH solution is used as a control. The aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were exhibited significant dose-dependent inhibition of DPPH activity. The DPPH radical inhibition was 54.54% and 95.36% at 120 µg/ml concentration (table 3). The result are correlated with previous studies that methanolic extract of *Alpinia galanga* showed effective inhibition of 67.78% DPPH antioxidant activity in a dose-dependent manner. The antioxidants present in the rhizome extract can acts as radical scavengers may protect the cells against various diseases such as cancer, neurodegenerative disorders [35].

Table 3: DPPH radical scavenging assay of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

Medicinal plants	Aqueous extract (µg/ml)	DPPH radical scavenging activity
Alpinia calcarata	20	13.06±1.54
	40	30.11±2.40
	60	42.04±5.62
	80	46.02±3.47
	120	54.54±4.43
Alpinia galanga	20	35.05±2.14
	40	54.63±4.18
	60	71.64±3.03
	80	86.59±3.96
	120	95.36±4.12

[a]Results are expressed as % of DPPH radical scavenging activity with respect to control. Each value represents mean±SD of the three determinants (n = 3)

Nitric oxide ($NO^{\bullet}$) radical scavenging activity

In this spectrophotometric method, the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured. NO, being a potent pleiotropic mediator in physiological processes and a diffusible free radical in the pathological conditions reacts with superoxide anion and form a potentially cytotoxic molecule, the peroxynitrite (ONOO). Its protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant. The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins in living systems. Nitric oxide (NO) is a chemical mediator and is involved in the regulation of various physiological processes generated by endothelial cells, neurons, macrophages, etc [36]. The maximum $NO^{\bullet}$ radical scavenging activity was 59.44% for *Alpinia calcarata* and was 73.10% for *Alpinia galanga* at 120 µg/ml concentration (table 4). Nitric oxide is generated in biological tissues by specific nitric oxide synthases [37]. The results correlated with the previous report showed that the ethanolic extract of *Alpinia calcarata* rhizome (EEACR) showed 72.89% of nitric oxide radical scavenging activity [38]. The previous literature reported that the significant radical scavenging activity may be due to the presence of antioxidant activity of flavanoid, which competes with O_2 and finally reacts with NO, leading to the lesser production of nitric oxide formation [39].

Table 4: Nitric oxide scavenging assay of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

Medicinal plants	Aqueous extract (µg/ml)	Nitric oxide scavenging activity
Alpinia calcarata	20	12.22±2.99
	40	23.21±3.67
	60	35.87±4.69
	80	49.31±2.43
	120	59.44±3.65
Alpinia galanga	20	16.25±3.46
	40	29.81±3.18
	60	47.34±2.55
	80	59.22±3.12
	120	73.10±2.30

[a]Results are expressed as % of Nitric Oxide scavenging activity with respect to control. Each value represents mean±SD of the three determinants (n = 3)

Fe^{3+}reducing power assay

The reducing power assay method is quantitative and hence it is expressed as ascorbic acid equivalents. The aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were exhibited significant dose-dependent reducing power activity. The presence of reductants/antioxidants in aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form (Fe^{2+}). Therefore, the Fe^{2+}can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. The maximum reducing property was found at the 120 µg/ml of aqueous extract of rhizomes of *Alpinia galanga* which was higher than the *Alpinia calcarata* (fig. 1). Researchers reported that phenolic compounds play a major role in scavenging free radicals by electron-transfer mechanism [40]. The reducing capacity of an extract may serve as a significant indicator of its potential antioxidant activity [41]. Researchers proved that the methanolic extract of *Alpinia pahangensis* showed the highest reductive activity due to the presence of antioxidants present in the rhizome extract of *Alpinia pahangensis*. The reducing power of the extract increased with the increase in the concentration of the extract until it reaches a certain level and then become constant. Basically, reducing power is associated with the presence of reductones that break the free radical chain by donating a hydrogen atom [42].

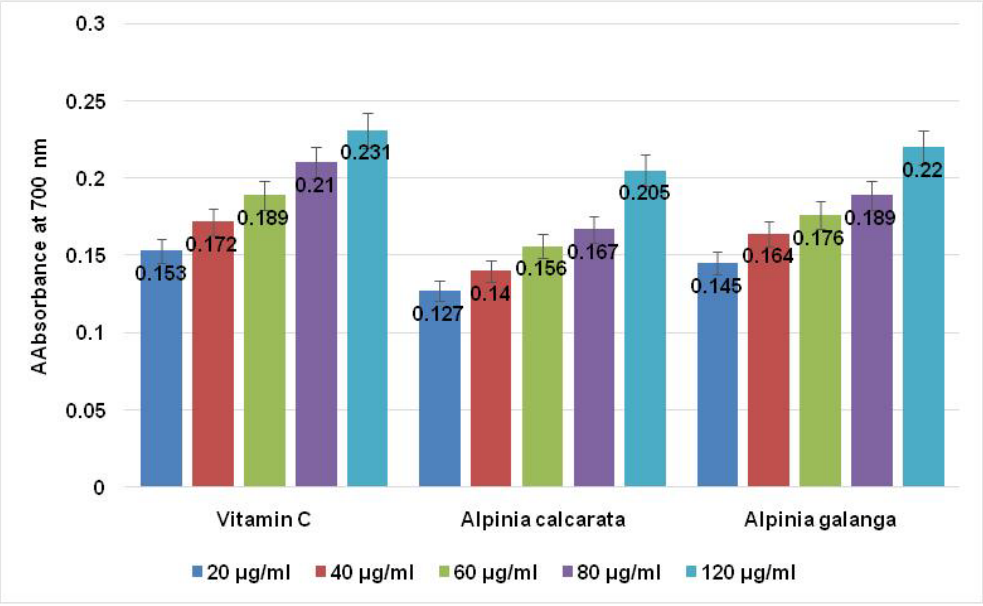

Fig. 1: Effect of rhizome of aqueous extract on reducing power activity of *Alpinia calcarata* and *Alpinia galanga*. The values represent the mean±SD of three determinants (n=3)

Phosphomolybdenum reduction assay

Phosphomolybdenum assay revealed that aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* have relatively potent reducing power. In the presence of a reducing agent, reduction of phosphomolybdic acid to phosphomolybdate blue ($Mo^{+6}$to Mo^{+5}) was observed [43]. The phosphomolybdenum assay method is expressed as ascorbic acid equivalents. In the concentration range from 20-120 µg/ml concentration, the aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* showed strong dose-dependent reducing activity.

The result obtained was confirmed by the high potency of the aqueous extract towards the transition metal ions. The aqueous extracts of rhizomes of *Alpinia calcarata* and *Alpinia galanga* were 55.47% and 78.38% respectively (table 5). Increase in absorbance of the reaction mixture indicates that the increase in reduction capacity of the extract. Antioxidants with DPPH radical scavenging activity donate hydrogen to free radicals, mainly lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and to form non-radical species, resulting in the inhibition of propagating phase of lipid peroxidation [44].

Table 5: Phosphomolybdenum reduction assay of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

Medicinal plants	Aqueous extract (µg/ml)	Phosphomolybdenum reduction assay
Alpinia calcarata	20	14.32±2.20
	40	24.16±2.43
	60	38.16±3.57
	80	43.95±3.29
	120	55.47±4.08
Alpinia galanga	20	23.53±2.38
	40	45.27±2.66
	60	57.30±4.21
	80	65.29±4.82
	120	78.38±5.13

[a]Results are expressed as % of Phosphomolybdenum reduction asssay with respect to control. Each value represents mean±SD of three determinants (n=3)

Anticancer activity of aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga*

The cytotoxic effect of aqueous extract of rhizomes of *Alpinia calcarata* and *Alpinia galanga* against human liver cancer cell line HepG2 was determined by a rapid colourimetric assay using MTT (methyl-thiazolyl-tetrazolium bromide) assay. In this study, rhizome aqueous extracts were tested for anticancer activity by MTT assay on HepG2 cell line. Measurements were performed and the concentration required for a 50% inhibition of viability (IC_{50}) was determined (fig. 2). The cytotoxic effect of rhizome aqueous extracts of HepG2 cells was expressed as the % cell viability. The morphology of HepG2 cells progressively changed from 7.8 µg/ml to 1000 µg/ml concentration of the extract and was compared with control. The maximum cell death was 73.59% for *Alpinia calcarata* and was 88.36% for *Alpinia galanga* was observed against human liver cancer cell line HepG2 cell line (fig. 3 and 4). The previous study suggests that the cytotoxicity of *Alpinia purpurata* extract on HeLa was evaluated by MTT assay based on a percentage of cell viability. The n-Hexane extract was found to have cytotoxic effect against HeLa, showing cell proliferation inhibition in a concentration-dependent manner. The IC50 for *A. purpurata* crude extract was found to be 41.25µg/ml [45].

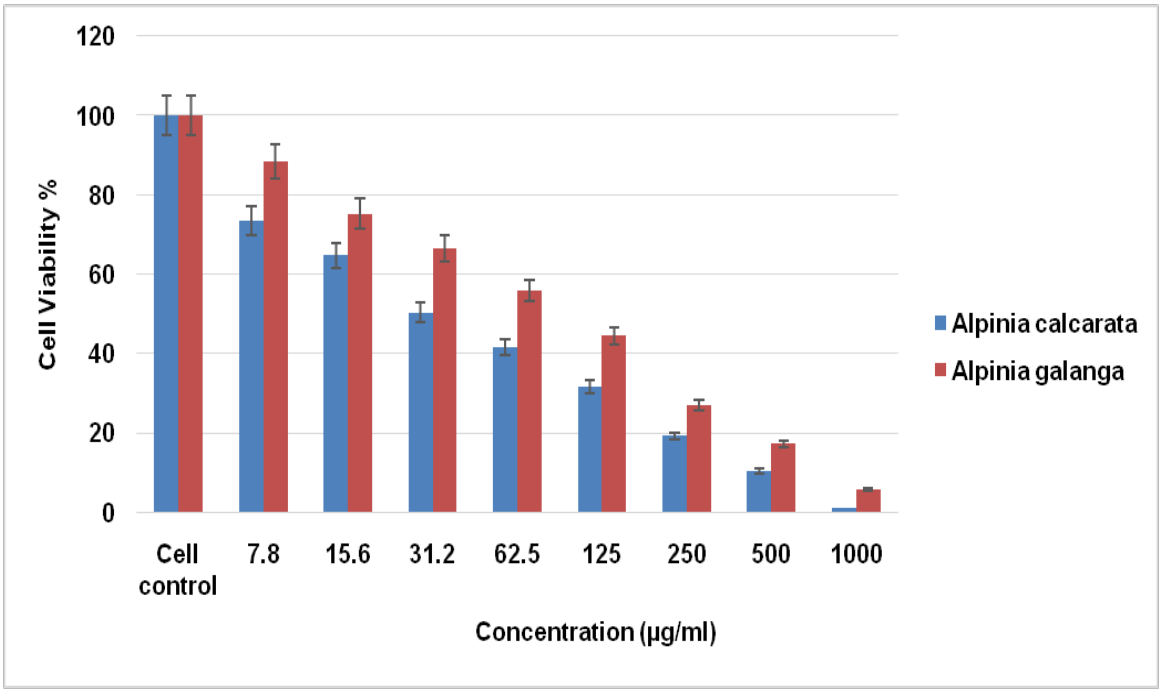

Fig. 2: Anticancer effect of the rhizome of aqueous extract of *Alpinia calcarata* and *Alpinia galanga* against HepG2 Human liver cancer cell line. Each data represents the mean from three independent experiments (mean±SD)

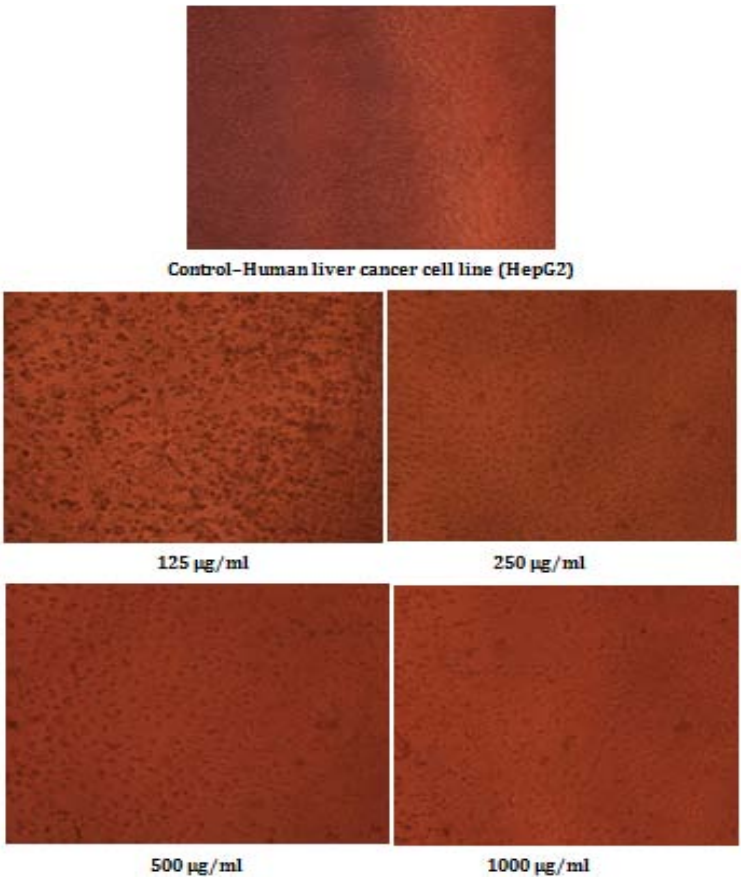

Fig. 3: Microscopic view of HepG2 cells of control and treated cells with aqueous extract of *Alpinia calcarata*. The morphology of HepG2 cells progressively changed from 7.8 µg/ml to 1000 µg/ml concentration of the extract and was compared with control

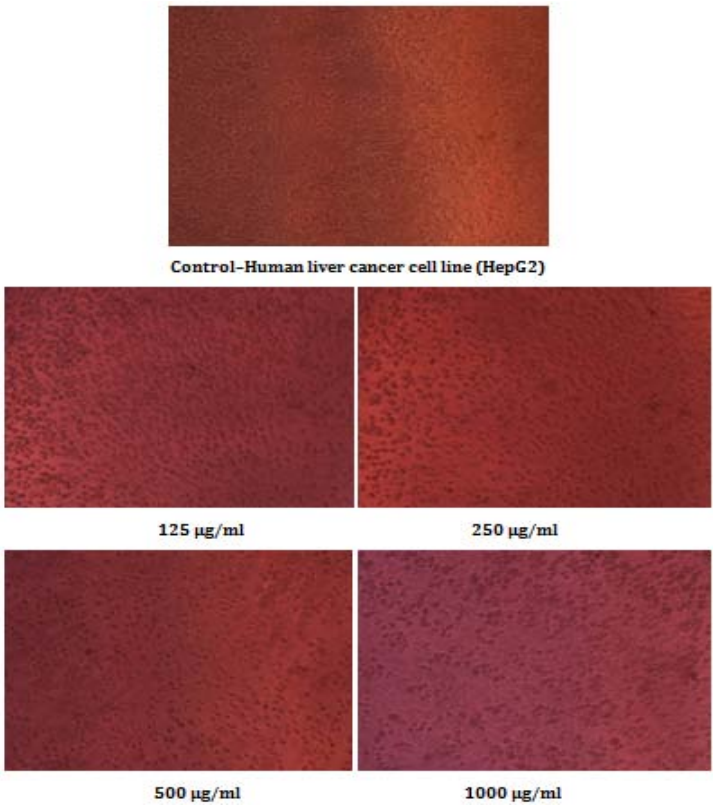

Fig. 4: Microscopic view of HepG2 cells of control and treated cells with aqueous extract of *Alpinia galanga.* The morphology of HepG2 cells progressively changed from 7.8 µg/ml to 1000 µg/ml concentration of the extract and was compared with control

CONCLUSION

Phytochemical screening of aqueous extracts *Alpinia calcarata* and *Alpinia galanga* rhizomes had revealed the presence of flavonoids, terpenoids, phenols, carbohydrates and proteins. The results of the present investigation indicate that *Alpinia galanga* rhizome extract exhibited the highest antioxidant activity in all the assays than *Alpinia calcarata.* The effect of aqueous extract of HepG2 cells are expressed as % cell viability. The results indicated that rhizome of aqueous extract of *Alpinia galanga* showed 88.36% cell viability whereas *Alpinia calcarata* showed 73.59% cell viability. Further investigation to be done for the identification of bioactive compounds from *Alpinia galanga* for the development of the anticancer drug.

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AUTHORS CONTRIBUTION

The design of the research work was done by the first author Dr. Vijayalakshmi Melanathuru. The experimental part of the work and writing of the manuscript was done by the corresponding author Dr. Sumathy rengarajan. The correction and the revision of the manuscript were done by Mr. Nithyanandan Thangavel.

CONFLICT OF INTERESTS

Declared none

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