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Phytochemical Evaluation and Anti-oxidant Activity of *Alpinia galangal* linn.

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KEYWORDS

Alpinia galanga, Phytocehmical Analysis, antioxidant, Free radicals scavenging.

ABSTRACT:

Introduction: The use of spices in the treatment of health problems has been a tradition in the world since early ages. *Alpinia galanga* is a well known medicinal plant in Southern Asia and has used for a long time. Because of its good aroma and taste, *Alpinia galanga* has been used widely for Asian cooking. *Alpinia galangal*is aromatic plants, enriched with bioactive compounds providing the usage for their therapeutic properties. In this study, the phytochemicals analysis, the anti-oxidant was done using Aerial parts extract of *Alpinia galanga*..

Objectives: To Investigate phytochemical evaluation on Aerial part of *Alpinia galanga* with various chemical tests of extracts and bioactive activity (anti-oxidant), in vitro assays such as Ferric reducing antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging techniques.

Methods: The material and method was done such as successive soxhlet extraction, preliminary phytochemical screening, qualitative tests for the identification of various active constituents viz. Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Protein and free amino acids, Saponins, Steroids, fixed oil, tannins and Phenolic, Inorganic chemicals etc, fluorescence analysis and Ferric reducing antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging techniques were used for anti-oxidant activity.

Results: Phytochemical investigations included successive soxhlet extraction; the obtained extractive values of aerial parts were found to be 2.64, 13.18 and 9.25 % from petroleum ether, methanol and aqueous solvent respectively. Preliminary qualitative phytochemical screening revealed the presence of flavonoids, carbohydrates, tannins & glycosides, fixed oil & fats along with few inorganic constituents i.e. iron, sulphate, phosphate and chloride. The DPPH scavenging activity was directly correlated with the concentration of the extract. The lower the IC_{50} value, the higher is the antioxidant activity, The FRAP results indicate that n-Hex and Methanolic fractions of *Alpinia galanga* may be useful for treating free radical related pathological damage.

Conclusions: *Alpinia galanga* belongs to the family Zingiberaceae with high medicinal values and used as traditional medicine. The whole plant is used for the treatment of various diseases. The Aerial part was dried and powdered and dissolved in alcoholic solvent. The study reveals that the extract contains many bioactive compounds like alkaloids, flavonoids, phenols, tannins, steroids and also exhibit anti-oxidant activity. Further in vivo studies on the extract will provide us better understanding of traditional claims of *Alpinia galanga* as medicinal herb.

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1. Introduction

The use of spices in the treatment of health problems has been a tradition in the world since early ages. As consumer preferences shift to natural products, the use of spices and natural aromatic plants as antioxidants and antimicrobials instead of synthetic food additives has been back on the agenda recently[1-2]. The importance of spices and aromatic plants has increased due to the side effects of synthetic drugs and the fact that bacteria can easily develop resistance to these synthetic drugs [2-4].

Alpinia galanga belongs to the family Zingiberaceae has been used as a traditional medicine in china for relieving stomach ache, treating cold, invigorating the circulatory systems and reduced swelling of the many chemical are among the characteristic compound which is known to possess antiplatelet, antioxidant, antiproliferative antiemetic activities[5]. Galangal is widely used to treat breathing diseases, stomach diseases, diarrhea, and stomach cramps. Galangal can also function as an antimicrobial replacement for antibiotics [6,7] Galangal is also effective for treating fever, abnormal menstruation, and increasing male fertility[8]. Galangal rhizome began to be used in several formulations to prevent cancer and tumors and is also used for the treatment of other diseases such as rheumatism, inflammation, diabetes, and neurological disorders[9-13]. Galangal is a mixture that has begun to be used by the community to overcome several chronic diseases[14].

A large number of phytochemical compounds, e.g., flavonoids, phenolic acids and volatile compounds, from several parts, such as leaves, rhizome and seeds, of *Alpinia galanga* have been analyzed and many potential phytochemicals have been reported [15-16]. Flavonoids of *Alpinia galanga* are continuously discovered from various parts of this medicinal herb [15-17]. The flavonoid phytochemical compounds from *Alpinia galanga* may possibly be an interesting alternative choice of bioactive molecules for cosmetic or cosmeceutical sectors—e.g., antioxidant skin care, anti-inflammatory cream/lotion and other botanicals[17].

2. Objectives

The present study is an attempt to study on *Alpinia galanga* Aerial part from Zingiberaceae family with a focus on new group of bioactive activity (anti-oxidant), which might have protective effects against cell oxidation. This was done by standardized novel in vitro assays such as Ferric reducing antioxidant power (FRAP) assay and 1,1-diphenyl-2-picrylhydrazil (DPPH) radical scavenging techniques and phytochemical investigation such as successive extraction and their various chemical tests with florescence.

3. Materials And Methods

Collection and authentication

Fresh whole plant was collected from the Manoj Nursery, Opposite T.V. Tower Dubagga, Hardoi Road, Lucknow, India in the month of December and the same was authenticated by the Birbal Sahni Institute of Palaeosciences, Lucknow. It was shade dried coarsely powdered.

Phytochemical Investigations

Extraction (successive soxhlet extraction):

The identified and authenticated plant was used for extraction process. About 2.5 kg of coarsely powdered aerial parts drug was subjected to successive extraction in a soxhlet apparatus with various solvents of increasing polarity (Petroleum ether, methanol & water). The individual extract was filtered, concentrated under rotary vacuum evaporator following on the boiling water bath to obtain sticky solid mass which was further dried in lyophilizer. The percentage yield of various extracts was calculated by the given formula. The results were as shown in Table No. 1.

% Yield = Amount of extract/ Amount of crude drug × 100

Preliminary Phytochemical Screening

Petroleum ether, methanolic and aqueous extracts of *Alpinia galanga* aerial parts were subjected to qualitative tests for the identification of various active constituents viz. Alkaloids, Carbohydrates, Glycosides, Phenolic compounds, Flavonoids, Steroids, tannins and Phenolic, Inorganic chemicals etc. The results were as shown in Table No. 2 & 3.

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A) Tests for Steroid [18]

Salkowski reaction: 2 ml of test solution was mixed with 2 ml of chloroform and 2 ml of conc. H_2SO_4 in a test tube and shaken well. The chloroform layer may appear red which indicate the presence of sterols and the acidic layer may show greenish yellow colored fluorescence to reveal the presence of steroid.

Liebermann - Burchard reaction: 2 ml of test solution was mixed with 2 ml of chloroform, 1-2 ml of acetic anhydride and 2 drops of conc. H_2SO_4 from the side of test tube which may be initially red then blue and finally green colour may appear.

Liebermann reaction: 3 ml of test solution was mixed with 3 ml acetic anhydride heated cooled and added with few drops of conc. H_2SO_4, blue colour may appear.

B) Tests for Glycosides [18]

i) Test for Cardiac Glycosides

Baljet's test: A thick section may show yellow to orange colour with sodium picrate.

Legal's test (Test for cardenoloids): To the aqueous and alcoholic extract 1 ml of pyridine and 1 ml of sodium nitroprusside was added, pink to red colour may appear.

Keller-Killiani test (Test for deoxysugars): 2 ml test solution was mixed with glacial acetic acid and added with 1 drop of 5 % $FeCl_3$ followed by addition of conc. H_2SO_4. Reddish brown colour may appear at the junction of the two liquid layers and upper layer may show bluish green colour which may correspond to the presence of glycosides.

ii) Test for Anthraquinone Glycosides

Borntrager's test for anthraquinone glycosides: 3 ml of extract was boiled with dilute H_2SO_4, filtered and then cooled. To the cooled filtrate equal volume of benzene or chloroform was added and then shaken well. The organic layer was separated and ammonia was added. The ammonical layer may become pink to red to ensure the presence of glycoside.

Modified Borntrager's test for C-glycosides: 5 ml of extract with 5 ml of 5 % $FeCl_3$ and 5 ml of dil. HCl was heated for 5 minute in boiling water bath, filtered and then cooled. To the cooled filtrate equal volume of the benzene or chloroform was added and then shaken well. The organic layer was separated and ammonia was added. The ammonical layer may show pinkish red colour to ensure the presence of glycoside.

C) Tests for Flavonoids [18]

Shinoda test: To the extract 5 ml of 95 % ethanol, few drops of conc. HCl and 0.5 g of magnesium turnings were added, pink colour may be observed.

With lead acetate solution: The small quantity of residue was taken with lead acetate solution which may show yellow coloured precipitate.

With aqueous NaOH solution: Small quantity of the extract was dissolved in aqueous sodium hydroxide and appearance of yellow color may indicate the presence of Flavonoids. The yellow colour may decolourise after addition of acid.

D) Tests for Alkaloids [18]

Small quantity of the extract was treated with few drops of diluted hydrochloric acid mixed well and filtered. The filtrate was used for the following tests.

Mayer's test (Potassium mercuric iodide solution): 2-3 ml of filtrate was mixed with few drops of Mayer's reagent; cream coloured precipitate may produced to indicate the presence of alkaloids.

Dragendroff's test (Potassium bismuth iodide solution): 2-3 ml of filtrate and few drops of Dragendroff's reagent were mixed in a test tube. A reddish brown precipitate may indicate the presence of alkaloids.

Hager's test (Saturated Picric acid): 2-3 ml of filtrate was mixed with few drops of Hager's reagent. A yellow colour precipitate may appear.

Wagner's test (Iodine in Potassium Iodide solution): 2-3 ml of filtrate was mixed with few drops of Wagner's reagent. A reddish brown precipitate may indicate the presence of alkaloid.

E) Tests for Tannins and Phenolic compounds [18]

Small quantity (2-3 ml) of the extract was mixed with ethanol and tests for the presence of phenolic compounds and tannins were carried out with the following reagents.

5 % FeCl3 solution: test solution may give deep blue-black colour that confirmed the presence of tannins.

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Lead Acetate solution: test solution may give white precipitate.

Gelatin solution: test solution may give white precipitate.

Acetic Acid solution: test solution may give red colour solution.

Potassium dichromate: test solution may give red precipitate.

Dilute Iodine solution: test solution may give transient red colour.

Dilute HNO_3: test solution may give reddish to yellow colour.

Dilute NH_4OH and potassium ferricyanide solution: test solution may give red colour solution.

Silver mirror test: one drop of NH_4OH and excess 10% $AgNO_3$ solution was heated with test solution for 20 min. on boiling water bath. White precipitate may show dark silver mirror deposits on wall of the test tube.

Dilute Potassium permanganate solution: test solution may show decolouration of the solution.

F) Tests for Inorganic Elements [18]

Ash of drug material was prepared and to this 50% v/v HCl or 50% v/v HNO3 was added. It was kept for 1 hour or longer and then filtered. With the filtrate,

following tests were performed and results shown in Table No. 2.

1) Test for Calcium: 10 ml of filtrate was mixed with 1 drop of NH_4OH and saturated ammonium oxalate solution. White precipitate of calcium oxalate may be soluble in HCl but insoluble in acetic acid.

2) Test for Magnesium: Calcium oxalate precipitate was filtered and separated from the above procedure. The filtrate was heated and then cooled with solution of sodium phosphate in dilute ammonia solution. White crystalline precipitate may indicate the presence of Mg.

3) Test for Potassium: 2-3 ml of test solution and few drops of cobalt nitrite solution were taken in a test tube. Yellow precipitate of potassium cobalt nitrite may appear.

4) Test for Iron:

a) 5 ml of test solution and few drops of potassium ferrocyanide were taken in a test tube which may show dark blue colour.

b) 5ml of test solution and few drops of 5% of ammonium thiocyanate (or 5% potassium thiocyanate solution) were mixed in a test tube. Blood red colour may appear.

5) Test for Sulphate:

a) 5 ml of test solution was mixed with few drops of 5% $BaCl_2$. White crystalline precipitate may indicate the presence of sulphate.

b) With the lead acetate reagent given white precipitate and then the precipitate may be soluble in NaOH.

6) Test for Phosphate: 5 ml of test solution was mixed with HNO_3 and few drops of ammonium molybdate solution, then heated for 10 min. and cooled. A yellow crystalline precipitate of ammonium phosphomolybdate may indicate the presence of phosphate.

7) Test for Chloride:

a) 3 ml test solution mixed with HNO_3 and few drops of 10% $AgNO_3$ solution was added. White precipitate of AgCl may appear which might be soluble in dilute ammonia.

b) 5 to 7 ml of filtrate was added with 3 to 5 ml of lead acetate solution. White precipitates thus obtained may be soluble in hot water which might show the presence of chloride.

FLUORESCENCE ANALYSIS [19]:

Fluorescence studies of shade-dried powdered aerial parts of *Alpinia galanga* plant was evaluated in which powdered drug was treated with different solvents i.e. 1-N sodium hydroxide in methanol, 1-N sodium hydroxide in water, 50% sulphuric acid, 50% nitric acid and observed under day light, short and long wavelength UV lights. The drug emitted various colour radiations and change in colour was recorded. The results were as shown in Table No. 4.

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ANTI-OXIDANT ACTIVITY

DPPH free radical scavenging assay

The antioxidant activity of the selected extracts was measured in terms of hydrogen donating ability 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical assay [20] in a reaction mixture containing different concentrations of extracts (2, 10, 20 and 50 µg/ml) and 1 mM methanolic solution of DPPH. Subsequently, the mixture was shaken vigorously and left to stand for 30 min in the dark.

Disappearance of the purple colour was monitored at 517 nm using spectrophotometer. Test samples and positive control ascorbic acid were tested in triplicate over the same range of sample concentrations. The radical scavenging activity was calculated as

% RSA= 100 (1-AE/AD),

Where

RSA is Radical Scavenging Activity

AE is the absorbance of solution containing antioxidant extract and,

AD is the absorbance of the methanolic DPPH solution.

The antioxidant activity of each test sample and ascorbic acid was expressed in terms of concentration required to inhibit 50% methanolic DPPH radical formation (IC50 µg/ml) and calculated from the graph of % RSA and plant extract concentrations.

Ferric reducing antioxidant power (FRAP) assay

FRAP assay was performed on the basis of the ability of the antioxidant to reduce ferric to ferrous ions in the presence of 2, 4, 6-tri (2-pyridyl)-S-triazine (TPTZ), forming an intense blue ferrous -TPTZ complex with absorption maxima at 593nm[21]. An assay mixture containing 2.5 ml of 30mM acetate buffer(pH 3.6), 0.25 ml of 10 mM TPTZ in HCl, 0.25 ml of 20 mM ferric chloride and different concentration of plant extract was incubated for 30 min at room temperature and the absorbance of the coloured product (ferrous tripyridyltriazine complex) was read at 593 nm. A standard graph for ferrous sulphate in methanol at different concentrations was prepared. FRAP values of the fractions were expressed as mM of Fe (II)/ g of extract.

4. Result And Discussion

In this study, the phytochemicals present in the alcoholic extracts of *Alpinia galanga* was analyzed using standard screening methods. The results reveal that the plant contains various bioactive constituents like phenols, tannins, alkaloids, flavonoids, steroids are present. The phytochemicals characteristics are summarize in the table – 3.

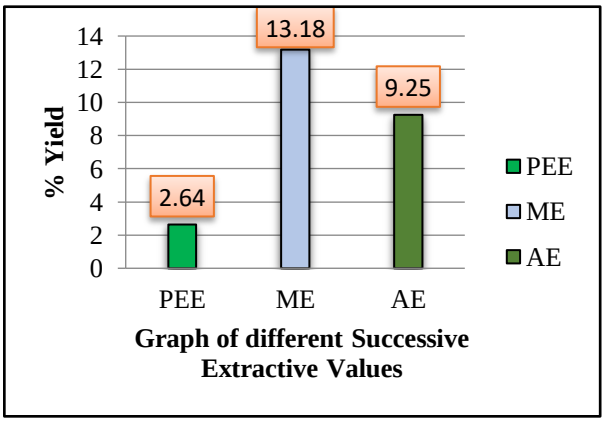
Phytochemical Analysis

Extractive values using successive extraction

Soxhlet extraction was performed with different solvents in increasing order of polarity, e.g. Petroleum ether, chloroform, ethyl acetate, methanol & water and percentage yields were calculated. Table No – 1.

Table – 1: Successive Extractive Values (Soxhlet Extraction) of Aerial parts of *Alpinia galanga*l.

Extracts	Aerial parts		
	% Yield (w/w)	Colour	Consistency
Petroleum ether	2.64	Yellowish green	Waxy
Methanol	13.18	Dark green	Sticky mass
Aqueous	9.25	Brown	Soft mass



Where PEE = petroleum ether extract, ME = methanolic extract, AE = aqueous extract.

The percentage yield of methanolic extract of aerial parts was found to be the highest as compared to petroleum ether and aqueous extracts.

The obtained percentage yield of successive extraction of aerial parts of *Alpinia galanga* was found to be 2.64

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(petroleum ether), 13.18 (methanolic) & 9.25 (water) respectively.

Preliminary Phytochemical Investigation:

Various extracts i.e. petroleum ether, methanolic and aqueous were subjected to preliminary phytochemical screening through qualitative chemical tests.

Detection of inorganic phytoconstituents

Various inorganic constituents i.e. iron, sulphate, phosphate & chloride were identified & the obtained results were as shown in table below.

Table – 2: Inorganic Elements of aerial parts of *Alpinia galanga*

Tests	Aerial parts (Powder drug)	
	HCl Treated	**HNO_3 Treated**
Calcium	-	-
Magnesium	-	-
Potassium	-	-
Iron	+	+
Sulphate	+	-
Phosphate	+	-
Chloride	+	-

Where ' + ' = Present, ' - ' = absent.

The inorganic phytochemical studies revealed that the aerial parts of the plant contained iron in both HCl and HNO_3 treated samples.

Detection of organic phytoconstituents:

Table – 3: Preliminary qualitative analyses (aerial parts) of *Alpinia galanga* extracts for the presence of various chemical constituents:

Test Name	Aerial parts		
	Petroleum ether	**Methanolic**	**Water**
Test for Steroids Salkowski test	-	-	-
Liebermann's Burchard	+	+	+
Liebermann's	+	-	-
Test for Glycosides For Cardiac Glycoside Baljet's test	-	+	+
Legal's test	-	+	+
Keller's killani test	+	+	-
Test for Anthraquinone Glycoside Borntrager's test	-	+	+
Modified Borntrager's test	+	+	+
Test for Flavonoids Shinoda test	-	+	+
Lead acetate	+	+	-
NaOH Test	+	+	+
Alkaloids Test Dragendorff's	-	-	-
Mayer's	-	+	-
Hager's	+	+	+
Wagner's	-	+	-
Murexide test for purine	-	-	-
Tannins and phenolic compound 5% $FeCl_3$	+	+	-
Lead acetate	-	+	-
Gelatin	-	-	-
Acetic acid	-	-	-
Potassium dichromate	-	-	-
Dil. Iodine	-	+	-
Dil. HNO_3	+	-	+
Dil. NH4OH and potassium ferricyanide	-	+	+
Silver mirror test	+	+	+
Dil. KMnO4	+	+	+

Where ' + ' = Present, ' - ' = absent.

The study revealed the presence of flavonoids, alkaloids, carbohydrates, tannins, steroids and glycosides as reported in table – 3.

Table – 4: Quantitative Fluorescence Analysis of Aerial parts powder of *Alpinia galanga*l.

S. No.	Treatment / Material	Day / Visible Light	Observation under U.V. Light (wavelength)	
			At Short(254nm)	**At Long (365nm)**
1	Drug Powder	Yellowish green	Yellow	Yellow
2	Rubbed powder	Yellowish green	Yellow	Yellow
3	In methanolic 1 N NaOH	Greenish yellow	Yellowish green	Yellowish green
4	In 1 N NaOH in water	Dark brown	Brown	Dark brown
5	In 1 N HCl	Off white	Off white	Pale yellow
6	In 50% HNO_3	Yellowish orange	No fluorescence	No fluorescence

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7	In 50% H_2SO_4	Black	No fluorescence	No fluorescence
8	In methanol	Greenish yellow	Dull green	Dull green
9	Water	Yellowish green	Greenish yellow	Greenish yellow
10	Chloroform	Yellowish green	Yellow	Yellow
11	Petroleum ether	Yellowish green	Yellowish green	Green
12	Dragendorff's reagent	Brownish black	No fluorescence	No fluorescence

Anti-oxidant activity

Antioxidants are the substances which protect the cells from caused by free radicals. They are produced in our body during break down of food or when exposed to tobacco smoke or radiations. These free radicals are one the important cause for heart disease, cancer and other diseases. The antioxidant potential of *Alpinia galanga* Aerial Parts extract was done by DPPH assay and FRAP assay. Since it a qualitative analysis, only the absorbance is measured. In FRAP assay, the production of green colour indicates the presence of antioxidants in the sample. In DPPH assay, the absorbance is measured at 517nm in UV-Visible spectrophotometer. The radical scavenging activity of the extract was determined as a decrease in the absorbance of DPPH. It is said that, lower the absorbance value, higher the scavenging activity of free radicals.

Table – 5: Antioxidant potential of various fractions of *Alpinia galanga*l

Fractions	DPPH IC50 (µg/ml)	FRAP value ± S.D. (µM Fe (II)/g of extract)
n-Hexane	35.62 ± 0.3	1500 ± 0.85
Methanolic	35.25 ± 0.4	1200 ± 0.62
Aqueous	>100	-
Vitamin - C	32.4 ± 0.2	1600 ± 0.35

DPPH free radical scavenging assay

The antioxidant activity of *Alpinia galanga* was assessed using the DPPH technique. The DPPH reagent (2, 2 Diphenyl 1-1-hydrazyl) is a stable radical that can be converted into a diamagnetic molecule by taking an electron or hydrogen radical. The decrease of DPPH in the presence of an antioxidant that donates hydrogen is the foundation of the DPPH technique. Extracts have a strong hydrogen-donating ability, which helps them to lighten DPPH's color. One of the substances that has a proton free radical with a distinctive absorption is DPPH, which is greatly reduced when exposed to substances that scavenge proton radicals [22]. The DPPH scavenging activity of *Alpinia galanga* was shown in Fig.2. The DPPH scavenging activity was directly correlated with the concentration of the extract. The lower the IC_{50} value, the higher is the antioxidant activity.

The data for IC_{50} for each fraction of *Alpinia galanga* is presented in Table – 5 and Figure –2. Lowest IC_{50} values were found to be for n-Hexane fractions (35.62 ± 0.3 µg/ml) which were comparable to the standard antioxidant Vitamin C (32.4 ± 0.2 µg/ ml) signifying the high proton donating and free radical scavenging potential of these fractions. The aqueous fraction did not show any appreciable antioxidant activity.

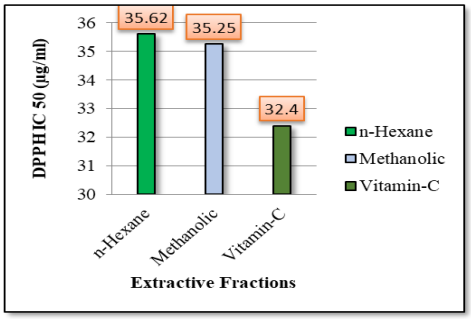

Figure – 2: DPPH free radical scavenging of various fractions of *Alpinia galanga* expressed as µg/ml required to scavenge 50% of free radicals.

Ferric Reducing Antioxidant Power (FRAP) assay

An intense bright blue Fe2+-TPTZ is created when a potential antioxidant interacts with a colorless Fe3+-TPTZ (tripyridyltriazine) complex. This process is known as the ferric-reducing antioxidant power test (FRAP). This technique worked well for reasonably priced compared with the efficacy of various compounds and screening of antioxidant capacities. As a result, in this study, the FRAP technique was used to examine the antioxidant capacity of chosen phenolic acids compounds [23]. Among all the tested fractions, n-Hexane fraction of *Alpinia galanga* exhibited highest FRAP Value (1500 ± 0.85 mM of Fe (II)/g) followed by

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the DCM fraction (1200 ± 0.62 mM of Fe(II) /g). In contrast the standard antioxidant Vitamin C had a FRAP value of 1600 ± 0.35 mM of Fe(II)/g. The results of FRAP assay are depicted in Table – 5 and Figure – 3. Flavonoids and other polyphenols due to their redox properties play an active role in quenching of free radicals. The results of the present study indicate that n-Hex and Methanolic fractions of *Alpinia galanga* may be useful for treating free radical related pathological damage.

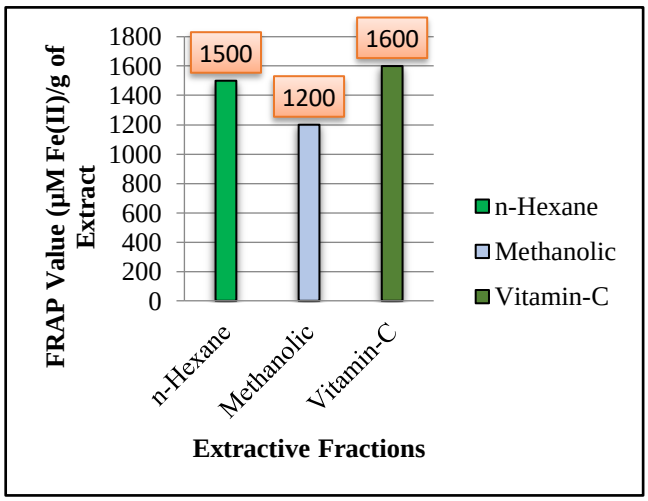

Figure – 3: FRAP antioxidant capacity of various fractions of *Alpinia galanga* expressed as mM of Fe (II) formed µ/g of extract.

CONCLUSION

Alpinia galanga belongs to the family Zingiberaceae with high medicinal values and used as traditional medicine. The whole plant is used for the treatment of various diseases. The Aerial part was dried and powdered and dissolved in alcoholic solvent. The study reveals that the extract contains many bioactive compounds like alkaloids, flavonoids, phenols, tannins, steroids and also exhibit anti-oxidant activity. Further in vivo studies on the extract will provide us better understanding of traditional claims of *Alpinia galanga* as medicinal herb.

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