

Antioxidant and Hepatoprotective Activity of Ethanolic Extract of *Alocasia indica* Tuber

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ABSTRACT

Alocasia indica (Araceae) is traditionally used in the treatment of abdomen and spleen related disorders. The edible tuber part of the plant is a very common vegetable among Indian people though maximum focus of research was done with the non-edible leaf part of the plant. In this study, ethanolic and aqueous extract of *A. indica* tuber were prepared to evaluate *in-vitro* antioxidant potential, *in-vivo* hepatoprotective activity and GCMS analysis of the extract. *In-vitro* analyses such as phenolic and flavonoid contents, antioxidant activity were done using standard spectrophotometric method and *in-vivo* antioxidant activity was evaluated by using carbon tetrachloride induced hepatic injuries in male *Albino Wister* rats. GCMS analysis was done by Perkin-Elmer Gas Chromatography-Mass Spectrometry. Biochemical analysis showed presence of various phytochemical compounds *e.g.* alkaloids, flavonoids, glycosides, saponin, tannins as well as SOD and catalase enzyme activity in both ethanolic and aqueous extract. Ethanolic extract showed higher phenolic, flavonoid content and antioxidant activity than aqueous extract. Ethanolic extract also have more potent antioxidant activity as evidenced by 2.5, 1.2, 1.13 and 1.42 fold lower IC₅₀ were found in the extract than aqueous extract for DPPH radical, hydroxyl radical, nitric oxide radical and superoxide radical scavenging activity respectively. GCMS analysis of ethanolic extract showed estra-1,3,5 (10)-trien-17-one, 3-hydroxy-6-methoxy, o-methyloxime as major component, which is phytosterol in nature. Ethanolic extract also showed potent hepatoprotective activity in *in vivo* study. The results indicated the potential use of strong antioxidant properties of *A. indica* tuber for therapeutic or pharmaceutical applications in future.

Keywords: *Alocasia indica*, Antibacterial activity, Antioxidant

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INTRODUCTION

Overproduction of reactive oxygen species (ROS, e.g. superoxide, hydroxyl, peroxy, and alkoxy radicals) and other free radicals by numerous physiological and biochemical processes is commonly referred to as 'oxidative stress'. The deleterious effects of ROS inside the body owes to their reaction and subsequent conjugation with biological macromolecules such as proteins, nucleic acids and lipids eventually leading to non-native protein forms, chromosomal aberrations and lipid peroxidation respectively. These in turn are responsible for serious ailments like cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases¹.

In India, the practice of Ayurveda (curing of ailments through herbal remedies) has been in vogue since ages. According to WHO, 80% of the population of developing countries who cannot afford synthetic pharmaceutical drugs rely on traditional plant based medicines in the treatment of various diseases with minimized side effects in some cases². This has necessitated the search for newer and more effective natural antioxidants of pharmaceutical use³. The presence of a wide range of phytoconstituents such as phenolics (flavonoids, phenolic acids and alcohols, tocopherols), thiols and caretonoids in medicinal plants protects the human body against oxidative damage by free radicals⁴.

Alocasia indica (Araceae) is widely cultivated in tropical and sub-tropical regions, with special mention to West Bengal, Assam, Maharashtra and Southern India. In local dialects of West Bengal, it is more popular as '*Mannkochu*'. The plant is a perennial herb frequently attaining heights of 5 meters. The tuber part of the *Alocasia indica* plant is edible and is also used as a common vegetable available easily and

cheap among general people. Since last few decades the plant is being used in the treatment of abdomen and spleen related disorders. The non-edible part of the plant, such as leaves, has been used as folk remedy for the treatment of various inflammatory ailments including rheumatism and bruises. It was also reported that the hydroalcoholic extract of the leaves of the plant contained free radical scavenging activity⁵, hepatoprotective activities⁵, antioxidant, antinociceptive, anti-inflammatory⁶, antimicrobial activities⁶ as well as antidiarrheal and *in vitro* antiprotozoal activities⁷. A preliminary study in the ethyl acetate and butanol extract of the root stalk of *A. indica* was also reported⁸ but no scientific data is available to validate the therapeutic use of the tuber part of the plant which is generally consumed as food. The objective of the present study is to perform a systemic evaluation of the antioxidant potential, free radical scavenging activity, phytochemical screening, hepatoprotective activity against CCl₄ damage and GCMS analysis of aqueous and ethanolic extracts of the edible tuber part of *A. indica*.

MATERIALS AND METHODS

Chemicals

Ethylenediamine tetraacetic acid (EDTA), ascorbic acid, 2-deoxy-2-ribose, trichloroacetic acid (TCA), nitro blue tetrazolium (NBT), reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulfate (PMS), sodium nitroprusside (SNP), sulfanilamide, naphthylethylenediamine dihydrochloride (NED), quercetin, 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) were obtained from Hi Media Research Laboratories Pvt. Ltd. Aluminium chloride (AlCl₃), ferric chloride (FeCl₃),

Sodium hydroxide (NaOH), hydrogen peroxide (H₂O₂), gallic acid, ascorbic acid, thiobarbituric acid (TBA), sodium nitrite were obtained from Merck, India.

Plant Material

The tuber vegetable (*Alocasia indica*) was collected from local market of Kolkata, West Bengal, India and was authenticated by the Botany Department of Serampore College, Hooghly, India. The tuber was chopped (2x2 inches) and dried under sun (40 ± 5°C) for a week. The dried tuber was finely powdered in grinder and sieved through the 40 micron sieve and stored in airtight containers.

Preparation of ethanolic (ETE) and aqueous (AQE) extracts of tuber

100 g of the dried and powdered tuber of *A. indica* was extracted in 500 mL of 80% (v/v) ethanol for 72 h in Soxhlet apparatus and the extract was centrifuged for 15 min at 4000 rpm. Supernatant was concentrated using rotary evaporator, residual water was dried in lyophilizer and was kept at -20°C for further use. Preparation of aqueous extract was done using water as solvent by following the same protocol by soxhlet apparatus as mentioned in preparation of ethanolic extract and dried in lyophilizer as above.

Estimation of dry extract weight (yield), protein and carbohydrate contents

Yield of the lyophilized dry tuber extracts (ETE and AQE) was measured. Total protein and carbohydrate contents were assayed by Bradford and orcinol-sulphuric acid⁹ reagents respectively.

Phytochemical screening

Phytochemical screening of ETE and AQE extracts of *A. indica* was carried out for detection of phytoconstituents like saponins, tannins, alkaloids, phlobatanins, glycosides etc¹⁰.

Determination of total phenolic content

The total phenolic content was determined by using the Folin-Ciocalteu reagent¹¹. About 1ml of tuber extract was mixed with 5 ml of Folin-ciocalteu reagent (1:10) followed by adding 4 ml of Sodium carbonate (0.7 M) and absorbance was measured at 760 nm. The concentration of total phenolic compounds was determined as mg gallic acid equivalents.

Determination of total flavonoid content

The total flavonoid content was determined with aluminium chloride (AlCl₃)¹². The tuber extract (0.1 ml) was added to 0.3 ml distilled water followed by NaNO₂ (0.03 ml, 5%) at 25°C. After 5 min, AlCl₃ (0.03 ml, 10%) was added and the reaction mixture was treated with 1 mM NaOH (0.2 ml). Finally, the reaction mixture was diluted to 1 ml with water and the absorbance was measured at 510 nm. Quercetin was used as standard.

Total antioxidant activity

Total antioxidant activities were determined by adding 0.3 ml of sample with 3.0 ml reagent solution prepared by mixing 0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate¹³. Reaction mixture was incubated at 95°C for 90 min and absorbance was measured at 695 nm. Total antioxidant activity was expressed as the number of equivalents of ascorbic acid in milligram.

DPPH radical scavenging activity

50 µl of 0.16 mM 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) solution in methanol was added to 50 µl of test sample or standard ascorbic acid. The mixture was kept in the dark for 30 min and measured at 517 nm¹³. The scavenging activity (%) was calculated by the following equation:

Scavenging activity (%) = [(Abs control – Abs sample) / (Abs control)] × 100(a)

Hydroxyl radical scavenging

The assay was performed by adding 0.1 ml EDTA (1mM), 0.01 ml FeCl₃ (10 mM), 0.1 ml H₂O₂ (10 mM), 0.36 ml deoxyribose (10 mM), 1.0 ml tuber extract or ascorbic acid (as standard, 1-10µg/ml), 0.33 ml phosphate buffer (50 mM, pH 7.4) and 0.1ml ascorbic acid (1 mM) in sequence¹¹. The mixture was then incubated at 37°C for 1h. 1.0 ml of the incubated mixture was mixed with 10% TCA (1.0 ml) and 0.5% TBA (1.0 ml) to develop the pink chromogen. Absorbance at 532 nm was measured and Percentage scavenging activity was calculated by using the formula (a) given above.

Nitric oxide radical scavenging

The extent of nitric oxide generation was measured using Griess reagent method¹². 4ml tuber extract (1-50 µg/ml) was added to 1ml of sodium nitroprusside solution (5mM) and incubated for 2 h at 27°C. 2 ml of the incubated solution was diluted with 1.2 ml of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride). The decreasing absorbance at 550nm was compared with standard ascorbic acid and scavenging activity was calculated using the above formula (a).

Reducing power

Reducing power was measured by adding 1.0 ml of different concentrations of ETE and AQE samples with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml (1%, w/v) potassium ferricyanide. Reaction mixture was incubated at 50°C for 20 min. Then 2.5 ml of TCA (10%) was added and centrifuged (at 650 g) for 10 min. From the upper layer, 2.5 ml solution was withdrawn and mixed with 2.5 ml distilled water and 0.5

ml FeCl₃ (0.1%) and increased absorbance was measured at 695 nm using BHT as standard¹³.

Superoxide radical scavenging and superoxide dismutase activity (SOD)

Appropriately diluted fractions of the sample were added to the reaction mixture containing 1 ml of NBT solution (156 µM prepared in phosphate buffer, pH 7.4), 1 ml of NADH solution (468 µM, prepared in phosphate buffer, pH 7.4). Finally, reactions were accelerated by adding 100 µl PMS solution (60 µM prepared in phosphate buffer, pH 7.4) to the mixture. After 5 min, absorbance at 560 nm was measured and results were compared with standard ascorbic acid (10-50 µg/ml)¹⁴. SOD activity was expressed as nKat/ min/ mg of protein.

Catalase activity

Catalase activity was determined by measuring the decrease in absorbance at 240 nm¹⁵. The reaction was started by adding 0.5 ml hydrogen peroxide (10 mM) in reaction mixture containing 0.5 ml tuber extract and 2.0 ml (0.1 M) sodium phosphate buffer (pH 6.8). Catalase activity was expressed as nKat/min/mg of protein.

Activity staining of SOD

For activity staining, 10% (w/v) native gel was run for 3 hrs at 40 mA at 4°C and the gels were stained with SOD native gel stain (NBT 2.43 mM, TEMED 28 mM and riboflavin-5'-phosphate 0.14 M in 50 mM phosphate buffer (pH 7.8)¹⁶. SOD activity was detected by a clear area (achromatic bands) against a dark purple background. A positive control was run in parallel to confirm the SOD activity.

Activity staining of Catalase

Catalase activity staining was done in 8% (w/v) native gel at 4°C with a positive control of catalase enzyme. The gel was

incubated with 0.003% H₂O₂ solution for 10 min and stained with 2% ferric chloride and 2% potassium ferricyanide solution¹⁶. A green-blue background with clear wide bands with no colour confirmed the presence of enzyme activity.

In vivo hepatoprotective activity

Studies were carried out using male Albino Wister rats weighing 140±10 g. The animals were housed in polypropylene cages in controlled temperature (22 ± 2°C) and normal day night cycle (12 h light and 12 h dark). All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC) of Indian Institute of Chemical Biology, Kolkata (Ref: IICB/AEC-APP/June-Meeting/2013). The animals were allowed to take standard laboratory feed and tap water during entire experimental procedure¹⁷. Rats were assigned to three groups, each consisting of 6 rats. Group I (the control group) received only vehicles; olive oil (0.5 ml/kg body weight) for 7 days. Group II were received oral administration of CCl₄ as a 50% solution in olive oil at 2 ml/kg body weight on day 1 and day 7 of the experiment. Group III received ETE (200 mg/kg body weight/day) intraperitoneally for 7 days along with the oral administration of CCl₄ on day 1 and 7. At the end of the experimental period, the rats were sacrificed by cervical dislocation. Blood was collected via cardiac puncture and subjected to preparation of serum. Biochemical analysis of serum marker enzyme including AST and ALT were measured using kits obtained from ACCUREX biomedical pvt. Ltd. Immediately after sacrifice liver was separated washed in ice cold saline and cut into small pieces for histopathological studies and biochemical analysis. For histopathological studies liver pieces fixed in formalin followed by routine method of haematoxylin-eosin or staining. For biochemical analysis liver pieces were

homogenized in 10% Tris HCl buffer (0.1 mole/L, pH 7.4) and phosphate buffer (pH 8, 0.01M) at 10000 rpm at 4°C for the estimation of lipid peroxidation and reduced glutathione (GSH) respectively.

Estimation of lipid peroxidation

Liver peroxidation was detected by measuring thiobarbituric acid reactive substance (TBARS). 2 ml of liver homogenate was mixed with 1ml of 20% (v/v) TCA and 1ml of 0.67% (v/v) TBA and then boil it for 10 minutes in a water bath. After cooling the mixture was filtered through Whatman filter paper and the reading of filtrate were taken at 530nm¹⁷. The amount of MDA was quantitated as an index of lipid peroxidation. The results were expressed as mmol MDA/mg protein.

Estimation of GSH

In 200µl PBS, 20µl of liver extract was mixed with 10µl dTNP (2, 2' dithiobis 5 nitro pyridine, 4mg/ml in methanol) mixed well and then incubate at room temperature for 15 minutes. After that readings were taken at 412nm¹⁷. Results were expressed as mmol GSH/mg protein.

Estimation of Protein

Protein in the homogenate of liver tissue was estimated by the method of Lowry using BSA as standard.

Gas Chromatography (GC)/Mass Spectrometry (MS) Analysis and Identification of components

Identification of the different compounds present in the extracts was performed using Polaris Q Mass spectrometer coupled with Trace GC ultragas chromatography (Thermo Fisher Scientific India Pvt. Ltd.) and investigated using Perkin-Elmer Gas Chromatography-Mass Spectrometry. A DB-5MS column (30 m x 0.25 mm) 0.25 µm film thicknesses with 5%

Phenyl polysilphenylene siloxane was used. Helium (He) (99.999%) was used as a carrier gas at a flow rate of 2 ml/min. 1 µl ETE sample was injected into the column in a split mode. The GC oven temperature was set initially at 50°C with a hold time of 1min and raised to 300°C at the rate of 80°C/min with a hold time of 5 min, raised to 320°C at the rate of 10°C/min with a hold time of 10min. The mass spectra were taken at constant EI ion source of 70 eV. The detector was set at 40-600 D and temperature was kept at 250°C. Interpretation of mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST) having 1,50,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The data store software XCALIBUR was used.

Statistical analyses

All data are given as the mean ± SD/SE of four to six measurements. IC₅₀ was calculated as the concentration which brought about 50% reduction in absorbance compared to blank. Kruskal-Wallis non parametric ANOVA and Mann-Whitney *U* multiple comparison test were performed in in-vivo study.

RESULTS AND DISCUSSION

Synthetic drugs are recently replaced by natural antioxidants due to its toxic effect in human body¹⁸. In the present paper, the *in vitro* antioxidant and antimicrobial potential of ethanol and aqueous extracts of tuber of *Alocasia indica* was evaluated along with GC-MS analysis of the extracts.

Yield, biochemical composition and phytochemical analysis of extract of *A. indica* tuber

Water extract (AQE) of the tuber showed 1.9 times higher yield than that of the

ethanol extracts (ETE) (Table 1A). The biochemical contents like protein and carbohydrate were quantified in crude extract powders. From AQE approximately 4.15 mg protein /g of tuber extract and 109 mg carbohydrate/g of extract were obtained respectively (Table 1A). In ethanol extract (ETE) the protein and carbohydrate contents were found to be respectively 2.21 times and 1.32 times higher than the AQE extract. Purification and characterization of a glycoprotein lectin reported with the tuber part of the plant which was needed for saccharide separation¹⁹.

Phytochemical screening of both the extracts (ETE and AQE) revealed the presence of alkaloids, flavonoids, tannins, glycosides and phenolics (Table 1B) as found in several plant extract^{20,21}.

Total phenolic and flavonoid content

It has been reported that phenolic compounds like flavonoids, phenolic acid, proanthocyanidins, diterpenes and tannins are derived from several medicinal plants²² and exhibit antioxidant activity by inactivating free radicals or by preventing the decomposition of hydroperoxides into free radicals^{23,24}. Flavonoids have free radical scavenging activity, inhibition of hydrolytic and oxidative enzyme activity as well as anti-nociceptive and anti-inflammatory activities^{6,25}. The results revealed that significant level of phenolic compounds (Figure 1A) and flavonoid contents (Figure 1B) were present in both the extracts of *A. indica* though the ethanolic extract possessed higher concentration of total phenolic compounds than the aqueous extract, which may be due to their respective polarities²⁶ and may provide antioxidant defence system in human body.

Total antioxidant activity and reducing power

Total antioxidant activity of ETE was higher than AQE (Figure 1C). ETE showed

higher antioxidant activity (729.69 ± 77.48 mg ascorbic acid equivalent) than AQE (369.78 ± 10.76 mg ascorbic acid equivalent) per gram of extract.

ETE (1 mg/ml) showed 12 fold higher reducing power than AQE (Figure 1D). It is known that ferric reducing power of bioactive compounds is associated with its antioxidant activity, which is evaluated by the transformation of Fe^{3+} to Fe^{2+} by antioxidants^{27,28}. Our study showed that the ferric reducing abilities of the ethanol and aqueous extracts of *A. indica* were enhanced by increasing their concentrations.

DPPH, Hydroxyl, Nitric oxide, Superoxide radical scavenging activity

Free radical scavenging effects of antioxidants are evaluated using DPPH, a free radical donor under *in vitro* conditions²⁹. Both ETE and AQE possessed DPPH scavenging activity that are capable of donating hydrogen to a free radical removing odd electrons responsible for free radical's reactivity³⁰. ETE of the tuber showed 3.5 times higher DPPH scavenging activity than AQE in 0.5 mg/ml of the extract (Figure 2A). The IC50 values of ETE (3.09 μ g/ml) and AQE (3.71 μ g/ml) were similar to the standard (2.85 μ g/ml) (Table 2). Hydroxyl radical is the most reactive among the ROS. In our study ETE showed higher hydroxyl radical scavenging activity as compared to the AQE and almost close to the standards. ETE of the tuber showed 3.5 times higher inhibition (%) than AQE in 0.5 mg/ml of the extract (Figure 2B). The IC50 values of ETE (3.09 μ g/ml) and AQE (3.71 μ g/ml) were similar to the standard (2.85 μ g/ml) in this assay (Table 2). Nitric oxide is produced in various physiological processes but excessive production is associated with various carcinomas and inflammatory conditions³¹. The nitric oxide, generated from sodium nitroprusside, reacts with oxygen to form nitrite. The tuber extracts inhibited nitrite

formation by directly competing with oxygen³². The present study proved that the ethanolic extract has more potent nitric oxide scavenging activity than the water extract (Figure 2C). IC50 value of the ETE and AQE were 13.97 mg/ml and 19.95 mg/ml respectively for nitric oxide scavenging activity (Table 2). Super oxide is very harmful to the cellular components in a biological system because it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals³³. The super oxide scavenging activity of both ETE and AQE increased markedly with the increase in concentration of the extracts but the activity was higher in ethanolic extract. At 1 μ g/ml, the percent inhibition by the ETE and AQE were 11.57% and 9.46% respectively whereas that of ascorbic acid was 38.04% (Figure 2D). IC50 value of ETE and AQE were 5.73 μ g/ml and 8.13 μ g/ml respectively, as compared to that of ascorbic acid (1.15 μ g/ml) (Table 2).

Quantification and staining of SOD and catalase activity

All human cells protect themselves against free radical damage by enzymes such as SOD and catalase³⁴. The superoxide scavenging activity of the tuber extract was also supported by the presence of superoxide dismutase (SOD) activity. Catalase is a major antioxidant defense enzyme that primarily catalyses the decomposition of H_2O_2 to H_2O and O_2 ^{30/35}. ETE contained 271.4 ± 19.95 nkat/min/mg protein of SOD activity and 1.38 ± 0.12 nkat/min/mg protein catalase activities whereas AQE contained 141.92 ± 20.95 nkat/min/mg protein of SOD and 3.27 ± 0.04 nkat/min/mg protein of catalase activity (Figure 3 A and B). Presence of the enzymes SOD and catalase were further confirmed by clear colourless bands in native gels run with ETE and AQE (Figure 3C and D).

Effect of ETE on serum AST and ALT levels

In vivo antioxidant activity was done by hepatoprotection against liver injury due to CCl₄ in rats which has been widely and successfully used by many investigators^{36,37}. CCl₄ is metabolized by cytochrome P-450 in endoplasmic reticulum and mitochondria with the formation of CCl₃O⁻, a reactive oxidative free radical, which initiates lipid peroxidation^{38,39} and enhanced marker enzymes such as ALT and AST⁴⁰. *In vivo* experiment showed that serum markers of ALT and AST level increased 2 fold (p<0.01) and 1.7 fold (p<0.01) by CCl₄ administered group as compared to normal group. Treatment with ETE at concentration of 200 mg/kg/day displayed the recovery percentage of serum ALT by 65.32% (p<0.01) and AST by 77.36% (p<0.01) when compared to CCl₄ treated group (Table 3).

Effect of ETE on MDA and GSH levels in liver homogenate

Malonaldehyde (MDA), hallmarks of lipid peroxidation, was markedly increased in ethanol-attenuated liver⁴¹.

In our experiment MDA was increased by 67.84% (p<0.01) in CCl₄ treated group than control group. ETE treated group at 200 mg/kg/day were able to recover MDA level by 41.39% (p<0.01). MDA is one of the end products in the lipid peroxidation process⁴². The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage⁴³. In our *in vivo* study elevation MDA level in CCl₄ treated animal indicate lipid peroxidation in liver tissue which was significantly reversed by ethanolic extract of *A. indica* tuber. Reduced glutathione is normally present in hepatocytes for detoxification of free radicals. GSH depletion increases the sensitivity of cells to various aggressions leading to tissue disorder and injury⁴⁴. In CCl₄ treated group there was a decrease in GSH level by 30.04% (p<0.001) compared to control group. ETE at

200mg/kg/day can enhanced the GSH level by 55.46% (p>0.001) (Table 3) can support the hepatoprotective activity of the extract by its potent antioxidant properties.

Histopathological changes of liver

CCl₄ induced severe necrotic changes and substantial changes in liver section⁴⁵ such as microvesicular steatosis, increase in sinusoidal space, inflammatory infiltration of lymphocytes, dilation of central vein and increase in fat droplet indicating early phases of liver injury was observed in CCl₄ treated group as compared to normal group (Figure 4 A and B). ETE at 200mg/kg/day can recover the changes (Figure 4 C) by showing the normal pattern of the central vein, radiating pattern of cell plates and absence of fat droplets as compared to CCl₄ treated group. Histopathological observations give an additional support of the effectiveness of the *A. indica* tuber extract in reducing liver injury caused by CCl₄ intoxication.

GCMS Analysis

GC-MS analysis of ETE showed the presence of sixteen bioactive compounds. The active components (Table 4) with their retention time (RT), molecular formula, molecular weight (MW), and concentration (peak areas %) are presented in Figure 13. The results demonstrated that the ethanolic extract of the tuber contained higher concentrations of flavonoids and phenolic compounds than the water extract. GCMS analysis of the ethanolic extract (Figure 5) identified presence of three major bioactive compounds of which 10,12,14-Nonacosatriynoic acid (peak area 15.3%) and Androst-4-en-11-ol-3,17-dione,9-thiocyanato (peak area 16%) were found as essential oil^{46,47} whereas the major peak 29.5 % Estra-1,3,5(10)-trien-17-one,3-hydroxy-6-methoxy,o-methyloxime was a phytosterol. These bioactive components can be purified and characterized in future.

CONCLUSION

In vitro and *in vivo* studies confirmed that the tuber of *A. indica* can be used as potential, inexpensive, safe and natural antioxidant for pharmaceutical applications. The ethanol extract of the tuber part of the plant contained higher phenolic and flavonoid content and scavenging activities as compared to those in the water extract. The ethanolic extract contained phytosterols as identified in GCMS analysis which must be purified in future for therapeutic uses.

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Table 1: Biochemical and phytochemical analysis of ETE and AQE of *A. indica* tuber

A: Estimation of dry extract weight, protein and carbohydrate

Parameter	ETE	AQE
Dry extract weight (mg/g dry weight of <i>A. indica</i> tuber)	0.94	1.79
Carbohydrate (mg/g dry extract)	144	109 (10.9 %, w/w)
Protein (mg/g dry extract)	9.16	4.15 (0.42 %, w/w)

B: Phytochemical screening

No.	Parameter	ETE	AQE
1.	Alkaloids	+	+
2.	Tannins	+	-
3.	Glycosides	+	+
4.	Saponins	+	+
5.	Flavonoids	+	+
6.	Steroids	-	-
7.	Phlobatanins	-	-
8.	Triterpenes	-	-

+ = phytochemical constituents are present.

- = phytochemical constituents are absent.

Table 2: IC₅₀ values of % inhibition studies of ETE and AQE of *Alocasia indica* tuber

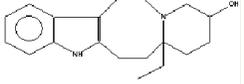
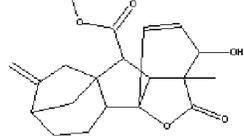
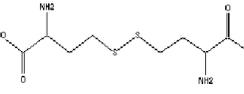
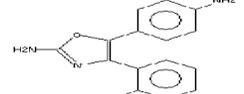
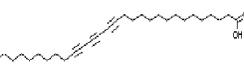
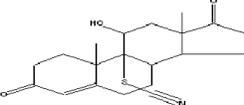
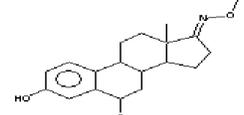
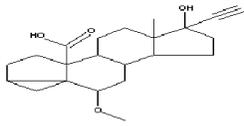
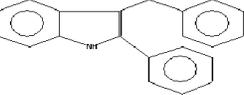
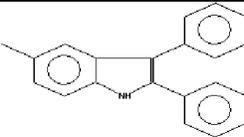
Parameters	IC ₅₀		
	ETE	AQE	Standard
DPPH radical scavenging	1.31 mg/ml	3.32 mg/ml	2.69 µg/ml
Hydroxyl radical scavenging	3.09 µg/ml	3.71 µg/ml	2.85 µg/ml
Nitric oxide radical scavenging	13.97 mg/ml	19.95 mg/ml	18.21 µg/ml
Superoxide radical scavenging	5.73 µg/ml	8.13 µg/ml	1.15 µg/ml

Table 3: *In vivo* hepatoprotective activities of ETE against CCl₄ induced liver damage

	Serum biomarker enzymes		Biomarkers in liver tissue	
	ALT ^{##} (U/L)	AST [#] (U/L)	MDA ^{##} (mmol/mg protein)	GSH ^{##} (mmol/mg protein)
C	22.66±1.78	25.16±1.64	53.95±2.34	6.39±0.17
CCl ₄	47.66±2.74*	44.33±4.46*	90.55±3.05*	4.47±0.17**
CCl ₄ + ETE	31.83±1.57*	29.5±1.52*	75.41±3.05*	5.535±0.16**

The values were the average of triplicate samples (n = 6) ± S.E. Significance level based on Kruskal-Wallis test: [#]p<0.01, ^{##}p<0.001. Significance level based on Mann-Whitney *U* multiple comparison tests. *p<0.01, **p<0.001

Table 4: Major components were identified in GCMS analysis of ETE

RT	Compound name and formula	% Area	MW	CAS#	Structure
4.23	β - Hydroxyque brachamine (C ₁₉ H ₂₆ N ₂ O)	1.9	298	NA	
4.54	Gibb-3ene-1, 10-dicarboxylic acid, 2, 4 α -dihydroxy -1- methyl-8methylene, 14 α -lactone, 10methyl ester (C ₂₀ H ₂₄ O ₅)	3.8	344	5508-47-4	
4.72	Butanoic acid, 4, 4 dithiobis (C ₈ H ₁₆ N ₂ O ₄ S ₂)	2.6	268	626-72-7	
4.81	5-(P-Aminophenyl)-4-(o-tolyl)-2 thiazolamine (C ₁₆ H ₁₅ N ₃ S)	8.4	281	NA	
5.04	3H-1,4-Benzodiazepine-2-amine,7- chloro-N-methyl-5-phenyl,4-oxide (C ₁₆ H ₁₄ CLN ₃ O)	4.7	299	58-25-3	
7.80	10, 12, 14 Nonacosatriynoic acid (C ₂₃ H ₄₆ O ₂)	15.3	426	NA	
7.94	Androst-4-en-11-ol-3, 17-dione, 9- thiocyanato (C ₂₀ H ₂₅ NO ₃ S)	16.0	359	1166-72-9	
8.34	Estra-1, 3, 5 (10)-trien-17-one, 3- hydroxy-6-methoxy, o-methyloxime, (6á) (C ₂₀ H ₂₇ NO ₃)	29.5	329	74299-26-6	
8.65	17 alpha-ethynyl-17 beta-hydroxy- 6- beta methoxy -3 alpha, 5 cyclo-5 alpha-androstan-19-oic acid (C ₂₂ H ₃₀ O ₄)	NA	358	NA	
9.47	1H indole, 3 benzyl -2-phenyl (C ₂₁ H ₁₇ N)	NA	283	52604-16-7	
9.96	1H indole, 5 methyl-2, 3 diphenyl (C ₂₁ H ₁₇ N)	NA	283	36804-50-9	

NA: Not Available

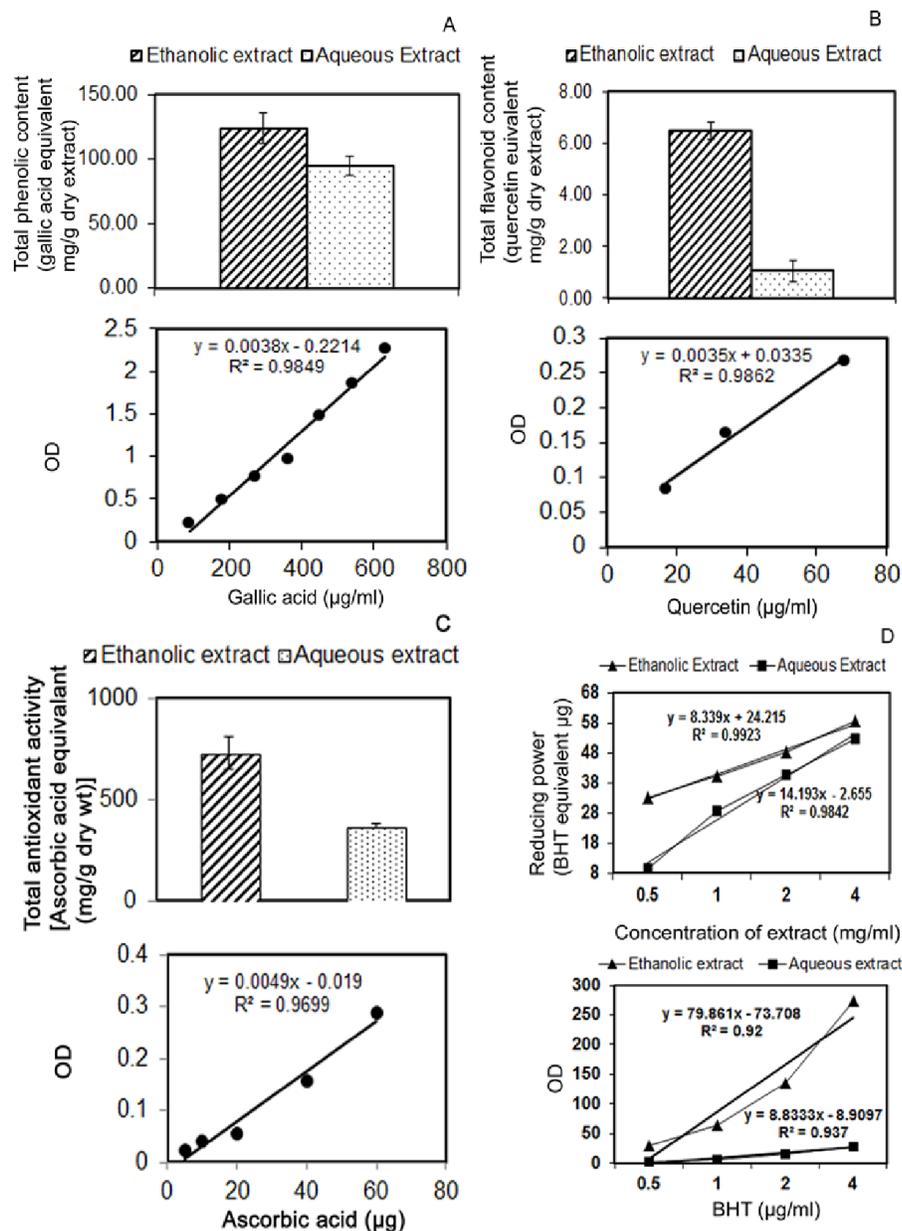


Figure 1. Total phenolic content (A), flavonoid content (B), total antioxidant activity (C) and reducing power (D) of ETE and AQE of *A. indica* tuber. Total phenolic and flavonoid contents were measured using gallic acid as standard. Total antioxidant activity was measured by ascorbic acid equivalent with ascorbic acid standard. Reducing power was measured using BHT as standard antioxidant.

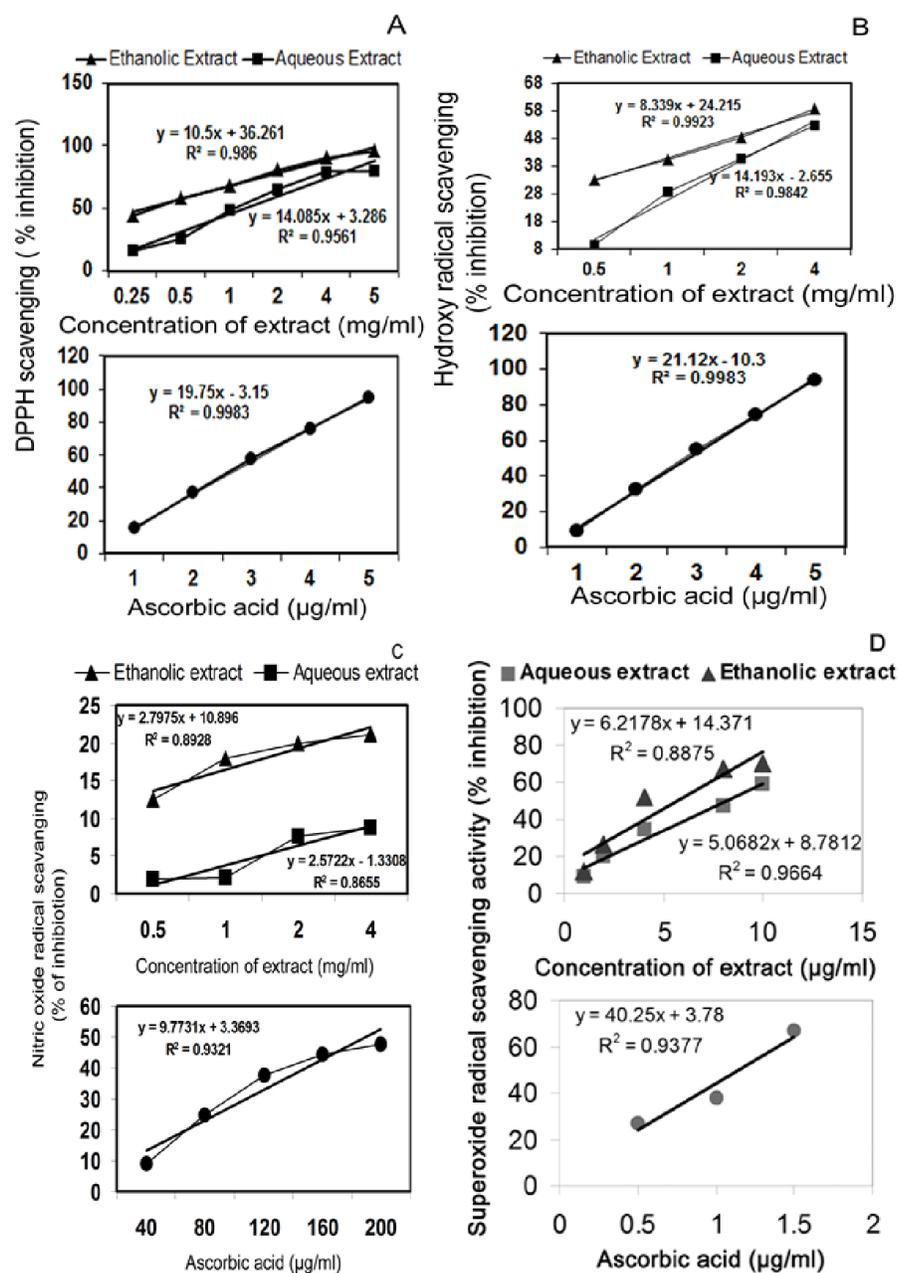


Figure 2. DPPH (A) Hydroxyl radical (B) Nitric oxide (C) Superoxide (D) radical scavenging assay of ETE and AQE of *A. indica* tuber using ascorbic acid as standard.

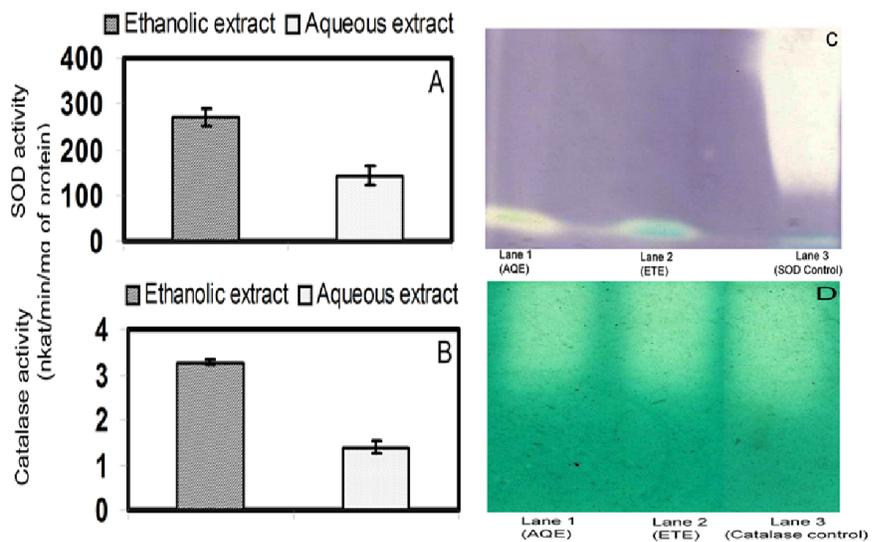


Figure 3. Quantification of SOD (A) and catalase (B) activities of ETE and AQE of *A. indica* tuber with identification of SOD (C) and catalase (D) by activity staining in native PAGE. Both the enzymes were identified as clear band in coloured background.

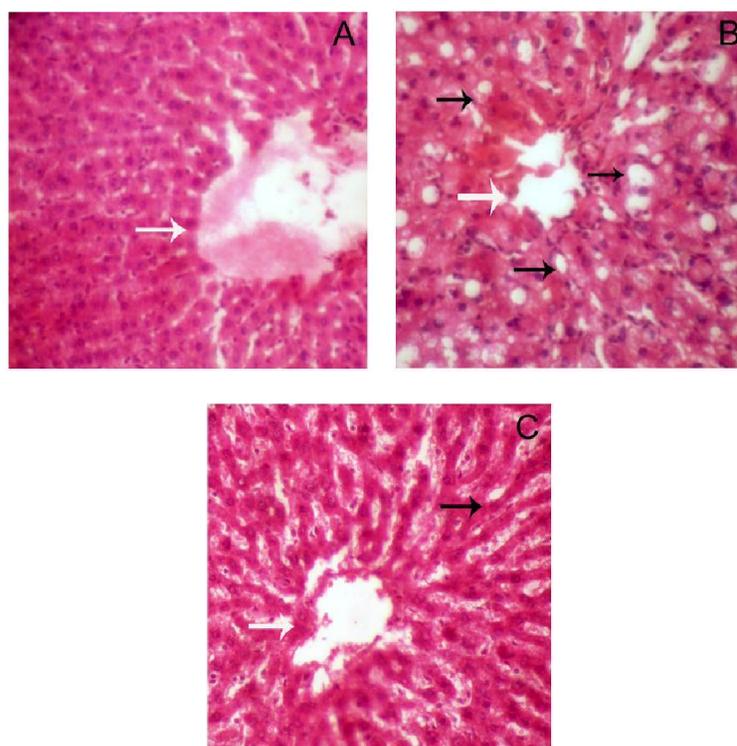


Figure 4. Hepatoprotection against CCl_4 induced liver damage. Micrograph of Haematoxylin-Eosin staining of control (A), CCl_4 treated (B) and CCl_4 with ETE at 200 mg/kg body weight/day (C) were taken at 400X. The black arrow indicates presence of lipid droplets and white arrow indicates the diameter of the central vein of the liver sections.

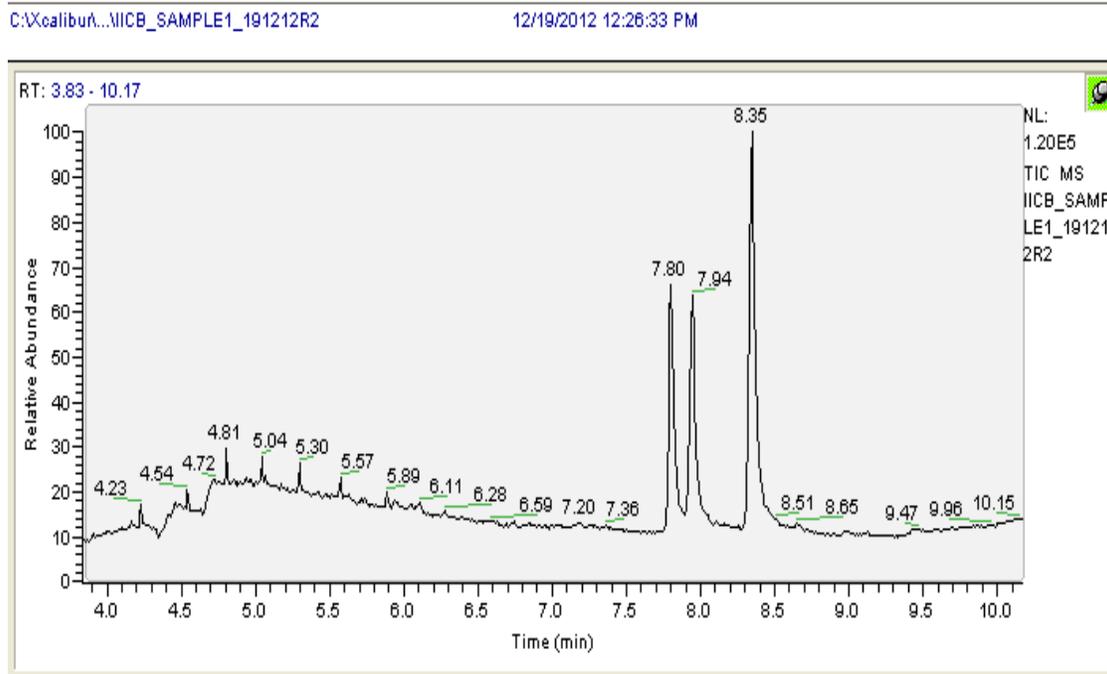


Figure 5. GCMS analysis of ETE of *A. indica* tuber



Figure 6. The plant *Alocasia indica* and the tuber part of the plant (inside the small box)