International Journal for Pharmaceutical Research Scholars (IJPRS)

V-3, I-1, 2014 ISSN No: 2277 - 7873

RESEARCH ARTICLE

Evaluation of Antioxidant, Antimicrobial and Cytotoxicity Activity of Hydroethanolic Extract and its Fractions of *Acorus calamus linn*

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Manuscript No: IJPRS/V3/I1/00031, Received On: 22/01/2014, Accepted On: 30/01/2014

ABSTRACT

Acorus calamus Linn which belongs to the family Araceae. Fractionation of hydro ethanolic extract was carried out by using solvent- solvent extraction. In the phytoconstituents analysis the petroleum ether fraction showed the presence of alkaloids, flavanoids, saponins, triterpinoids, phenolic, protein and carbohydrates, chloroform fraction showed the presence of flavanoids, saponins, triterpinoids, phenolics, carbohydrates, the ethyl acetate fraction showed the presence of alkaloids, saponins, triterpinoids, glycosides, the acetone fraction showed the presence of alkaloids, saponins, triterpinoids and glycosides, the aqueous fraction showed the presence of alkaloids, flavanoids, triterpenoids, glycosides, phenolics and carbohydrate. The ethyl acetate fraction showed a considerable increase in the total phenolic content than those of the crude extract. Chloroform and ethyl acetate fractions have shown relatively good antioxidant potential in DPPH, ethyl acetate fraction showed good antioxidant property in ABTS method, alkaline DMSO Method, Hydrogen peroxide radical scavenging activity, and total antioxidant capacity. Chloroform and ethyl acetate fractions showed relatively good antibacterial potential at a concentration below 500 µg/ml against both gram positive and gram negative microorganisms, by cup plate method, the zone of inhibition ranging from 13 to 25mm diameter. The chloroform fraction showed a moderate antifungal activity at a concentration of 500 µg/ml, by cup plate method, the zone of inhibition was found to be 12 to 19 mm diameter. MIC for ethyl acetate and acetone fractions was found to be less than 500 µg/ml when compared to the crude extract and the other fractions. In cytotoxicity studies, chloroform fraction showed cytotoxicity against the MCF-7 cells with CTC_{50} value of 110 µg/ml, whereas the rest of the fractions were moderately cytotoxic to the MCF-7 cell line with CTC_{50} values ranging from 170- 360 µg/ml.

KEYWORDS

Acorus Calamus, Antioxidant, Antimicrobial, Minimum inhibitory concentration, Cytotoxicity

INTRODUCTION

Acorus calamus linn which belongs to the family Araceae. In English it is called as Sweet flag, in Malayalam it is called as Vayanbu and in Tamil it is called as Vasambu.

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Acorus calamus is a native of eastern countries and also it is indigenous to marshes of the mountains in India, mainly in Himalayas and Sikkim at altitude of 2000m. *Acorus calamus* Linn, is a perennial herb with long branched cylindrical rhizome which is ¾ inch in diameter smooth pinkish or pale green. Its leaf scars are brown white and spongy.

It possesses slender roots. Its leaves are few. A strongly aromatic semi aquatic perennial herb; rhizomes creeping, jointed, somewhat vertically compressed, 1.3 to 2.5 cm thick, pale to dark brown and spongy inside. Leaves narrow up to 80 cm long, linear to narrowly enciform, glossy bright green, apex acute, petioles sheathing for 20 to 50 cm long. Fruits green angular, 3 celled, fleshy, containing 1 to 3 oblong seeds. Sweet flag has a very long history of medicinal use in Chinese and Indian herbal traditions[1]. The rhizomes and floral parts emit volatile compounds which could be used as scents[2]. It is widely employed in modern herbal medicine as well as its sedative, laxative, diuretic and carminative properties. Both roots and leaves of *Acorus calamus* have shown antimicrobial and insecticidal activities. Roots contain tricyclic sesquiterpine-callaminon, aclamendiol and isocalmendiol. Rhizome and root contain volatile oil where asarone and beta asarone are the important constituents.

It also contains α pinene, calamenol calamine, calamenone, eugenol, methyl eugenol, calamine, azulene, sugars, glucosides and flavones. It also contains bitter amorphous principle acorine. The plant has reported to have effect on central nervous system[3], metabolism mediated interactions with CYP 3A4 and CYP2D6[4], anti-inflammatory[5,7], inhibition of adipogenesis[6], insulin sensitizing activity[8], broncho dilatory effect[9], inhibitory effect of anaphylactic reactions[10], neuroprotective effect[11], antioxidant and DNA damage protection[12,13], antidiabetic[14,15], immunosuppressive properties[16], mitogenic activities[17], antidepressant activity[18], antihypertensive activity[19], inhibit morphogenesis.[20]

It is also reported to have antimicrobial, antiviral, analgesic, anticonvulsant, antidiarrheal, antirheumatic, diaphoretic, diuretic, emetic, expectorant, reduces fever, also used in insomnia, neurosis, epilepsy, hysteria and loss of memory.[21] This paper deals with the antioxidant and antimicrobial properties of various fractions that has been obtained from the hydro alcoholic extract of *Acorus calamus*.

MATERIALS AND METHOD

Collection of Plant Materials

Fresh rhizomes of *Acorus calamus* were collected from the survey of medicinal plants and collection unit, government Arts College, Ooty, Tamilnadu, authenticated by S. Rajan, Botanist, Udhagamandalam and a voucher specimen was preserved for further reference.

Preparation of Plant Extract and Fractionation[22]

The plant material collected was cleaned off adulterant, which include; scale attached to the rhizome and adventitious roots and coarsely grounded. The powdered material was soaked in 70% aqueous methanol at room temperature at 23-25ºC for 3 days with occasional shaking. It was filtered through a muslin cloth and then through a filter paper (Whattman qualitative grade 1). This procedure was repeated thrice and the combined filtrate was evaporated on rotary evaporator at 35-40ºC under reduced pressure (-760mm Hg) to a thick, semi-solid mass of dark brown color.

Solvent-Solvent Fractionation[22]

Activity directed fractionation of the crude extract was carried by standard phytochemical procedures using different organic solvents. A known quantity (10g) of the extract was dissolved in hydro ethanol (water and Ethanol in the ratio 1:1). This was then introduced in a separating funnel, petroleum ether (50-70ml) was then added and the mixture was shaken vigorously with regular allowing the air to escape out. It was kept for about 30 min to let the two layers to separate. The upper layer of petroleum ether was acquired and the same procedure was repeated twice and all petroleum ether layers are collected and concentration a rotary evaporated to obtain the petroleum ether fraction. Same procedure was repeated with chloroform, ethyl acetate, acetone and water respectively.

Qualitative Phytochemical Analysis[22,23,24,25,26]

The samples fractionated from the hydro ethanolic extract of *Acorus calamus* by solvent -

solvent fractionation where subjected to various chemical tests for the identification of various chemical constituents such as carbohydrates, alkaloids, steroids and sterols, glycosides, flavonoids, tannins, proteins and amino acids that present in each fraction. Quantitative phytochemical analysis was also carried out for the flavonol and phenol content by standard procedures.

Antioxidant Screening

Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging Method [27]

96 well plates were used to carry out this assay. 10 µl of each of the test sample or standard solution was added separately in each well of the microtitre plate which contains 200 µl of DPPH solution. The concentrations in the range of 1.95- 1000 µg/ml were used in this study. After the addition of all the samples and reagents the plates were incubated at 37ºC for 20 minutes and the absorbance of each solution was measured using an ELISA plate reader against the corresponding test and standard blanks and the remaining DPPH was calculated. The minimum concentration (Inhibitory concentration) of the sample required to scavenge 50% of the DPPH radicals is represented as IC_{50}.

Formula

$$IC_{50} = \{control - sample/control\} \times 100$$

ABTS Radical Scavenging Method[28]

To 0.2 ml of various concentration of the extract or standard, 1ml of distilled DMSO and 0.16 ml of ABTS solution were added to make a final volume of 1.36 ml. Absorbance was measured spectrophotometrically, after 20 min at 734 nm using ELISA reader. Blank is maintained without ABTS. IC50 value obtained is the concentration of the sample required to inhibit 50% ABTS radical mono cation.

Scavenging of Superoxide Radical by Alkaline DMSO Method[29]

To the reaction mixture containing 1ml of alkaline DMSO, 0.3 ml of each fraction in DMSO at various concentration were added to 0.1 ml of NBT (0.1 mg) to give a final volume of 1.4 ml. the absorbance was measured at 560 nm.

Scavenging of Hydrogen Peroxide Radicals[30]

A solution of H_2O_2 (20mm) was prepared in PBS, (pH7.4). Various concentrations of 1ml of the fractions or standard in methanol were added to 2ml of h_2o_2 solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution in PBS. The absorbance was measured at 230 nm, after 10 min against a blank solution that contained extract.

Antimicrobial Activity

Microorganisms used for antimicrobial studies:

In Gram positive microorganisms, *Bacillus coagulans Bacillus cereus Staphylococcus aureus, Bacillus subtilis*

In Gram negative, *Eschericia coli, Pseudomonas aerogenosa, Klebsiella pneumonia*

In fungi, *Candida albicans, Aspergillus niger, Sacchromyceas cerviasiae*

Cup Plate Method[24, 25]

Sterile nutrient agar and Sabourd dextrose agar plates where prepared, by pouring the sterile agar into sterile petri dishes under aseptic conditions. 1 ml of test organisms were spread over the agar plates. 5mm diameter holes were made in the agar plates using a sterile borer. Drug as well as the standard drug and the DMSO solvent control were added into each hole separately. The plates were maintained at $+4^0$C for 1 hour to allow the diffusion of solution into the agar. The plates are kept upright and incubate at 37^0C for 24 hours for bacteria and 28^0C for 48 hours for fungi. After the incubation period the zone of inhibition was measured.

Determination of Minimum Inhibitory Concentration (MIC) in Liquid medium[24, 25]

A series of test tubes were prepared containing the same volume of medium inoculated with the test organisms. Decreasing concentration of

drug were added to the tubes, usually a stepwise dilution by a factor 2 (two fold serial dilution) was used. (i.e. If the concentration is 500mg/ml, in the second tube it will be 250µl/ml and so on). One tube was left without drug, to serve as a positive control for the growth of the organism. The cultures were incubated at a temperature optimal for growth of the test organism for a period sufficient for the growth of the organism. The cultures were incubated at a temperature optimal for the growth of the test organism for a period sufficient for the growth of at least 10-15 generations. The cultures were incubated at temperatures optimal for growth of the test organism for a period sufficient for the growth of at least 10-15 generations (Usually 24 hours for bacteria and 48 Hours for fungi at 27^0C). The tubes are inspected visually to determine the growth of the organism indicated by turbidity. (in fact, turbidity of the culture medium is indicative of the presence of a large number of cells). The tubes containing the antimicrobial agent in concentration sufficient to inhibit the growth remain clear. In experimental terms the MIC is the concentration of the drug present in the last clear tube. i.e. The tube having the lowest antibiotic concentration in which growth is not observed.

Cytotoxicity Studies [26,27,28]

Determination of Total Cell Protein Content by Sulphorhodamine B (SRB) Assay

SRB is a bright pink amino xanthine dye with two sulphonic groups. Under mild acidic conditions, SRB binds to protein basic amino acid residues in Trichloro acetic acid (TCA) fixed cells to provide a sensitive index of cellular protein content that is linear over a cell density range of atleast two orders of magnitude.

Colour development in SRB assay is rapid, stable and visible. The developed colour can be measured over a broad range of visible wavelength in either a spectrophotometer or a 96 well plate reader. When TCA-fixed and SRB stained samples are air dried, they can be stored indefinitely without deterioration

Procedure

The monolayer cell culture was trypsinized and the cell count was adjusted to 1.0 x 10^5 cells/ml using medium containing 10% new born calf serum. To each well of 96 well microtitre plates, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once with medium and 100µl of different drug concentrations were added to the cells in the microtitre plates. The plates were then incubated at 37ºC in 5% CO_2 atmosphere, and the microscopic examination was carried out and the observations recorded every 24 hrs. After 72 hrs 50 µl of 50% Trichloro acetic acid was added to the wells gently such that it forms a thin layer over the drug dilutions to form an overall concentration of 10%. The plates were incubated at 4ºC for one hour. The plates were flicked and washed five times with tap water to remove traces of medium, drug and serum, and were then air dried. The air dried plates were stained with SRB for 30 minutes. The unbound dye was then removed by rapidly washing four times with 1% of acetic acid. The plates were then air dried. 100µl of 10m M Tris base was then added to the wells to solubilize the dye. The plates were shaken vigorously for 5 minutes. The absorbance was measured using micro plate reader at a wavelength of 540nm. The percentage growth of inhibition was calculated using the formula below

% Growth Inhibition = 100-{Mean OD of Individual Test / Mean OD Control Group} 100

RESULTS AND DISCUSSION

Percentage yield of crude hydro ethanolic extract was found to be 20% Percentage yield of different fractions of *Acorus calamus* hydro ethanolic extract are shown in the table 1.

Table 1: Percentage yield of Different Fractions

Sl No	Solvent	% yield
1.	Petroleum ether	11
2.	Chloroform	10
3.	Ethyl acetate	12
4.	Acetone	13
5.	Water	16

Phytochemical Studies

Qualitative Phytochemical Analysis

The crude hydro methanolic extract of *Acorus calamus* and its different fractions were subjected to various chemical tests as per the standard methods for the identification of various constituents. Qualitative phytochemical analysis of the crude extract of *Acorus calamus* showed the presence of alkaloids, flavonoids, saponins, triterpinoids, glycosides, steroids, phenolics, proteins and carbohydrates; and absence of tannins and aminoacids.

The different fractions were also subjected to various chemical tests for the identification of various constituents.

The petroleum ether fraction showed the presence of alkaloids, flavanoids, saponins, triterpinoids, phenolic, protein and carbohydrates.

The chloroform fraction showed the presence of flavanoids, saponins, triterpinoids, phenolics and carbohydrates and absence of alkaloids, tannins, glycosides, steroids, proteins and aminoacids.

The ethyl acetate fraction showed the presence of alkaloids, saponins, triterpinoids, glycosides and phenolics and absence of flavanoids, steroids proteins, aminoacids, carbohydrates and tannins.

The acetone fraction showed the presence of alkaloids, saponins, triterpinoids and glycosides and absence of flavanoids, tannins, steroids, phenolics, proteins, amino acids and carbohydrates.

The water showed the presence of alkaloids, flavanoids, triterpenoids, glycosides, phenolics and carbohydrate and absence of saponins, tannins, steroids, proteins and amino acids.

The results from the chemical tests are summarized and shown in table: 2

Quantitative Phytochemical Analysis

Estimation of Total Phenolic Content

The crude hydro ethanolic extract of *Acorus calamus* rhizomes and its different fractions were subjected for their estimation of total phenolic content and were expressed as mg gallic acid equivalent. The total phenolics of the extract and its fractions are presented in the figure.1

The amount of total phenolics in the crude extract was observed to be 40.2±1.934 mg gallic acid/g dry weight. The amount of total phenolics in different fractions; petroleum ether, chloroform, ethyl acetate, acetone and water were comparable with their observed values 21±2.387, 26±2.287, 43±1.871, 15±1.140, 32±2.588 mg gallic acid /g respectively. The ethyl acetate fraction showed a considerable increase in the total phenolic content than those of the crude extract which may be due to the recurrent purification during the fractionation.

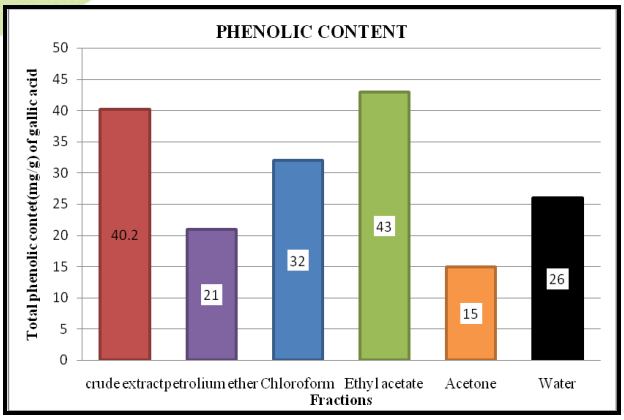

Figure 1: Estimation of Phenolic Content

Estimation of Total Flavanoids

The crude hydroethenolic extract of *Acorus calamus* and its fractions were subjected for their estimation of total flavanoids by aluminum chloride colorimetric method and absorbance of reaction mixture was measured at 415 nm.

Table 2: Qualitative Phytochemical Analysis

Tests	Crude extract	Pet ether	Chloroform	Ethyl acetate	Acetone	Water
Alkaloids	++	+	-	+	+	+
Flavaoids	+	+	+	-	-	+
Saponins	++	+	+	+	+	-
Triterpinoids	++	+	+	+	+	++
Tannins	-	-	-	-	-	-
Glycosides	+	-	-	+	+	+
Steroids	+	-	-	-	-	-
Phenolics	++	+	+	+	-	++
Proteins	+	-	-	-	-	-
Amino acids	-	-	-	-	-	-
Carbohydrate	+	+	+	-	-	+

The amount of total flavanoids in crude extract was observed to be 25 ± 1.581 mg/g rutin. Among the five fractions chloroform fraction showed relatively high amount of flavanoid content; 26 ± 2.827 mg/g rutin compared to other fractions.

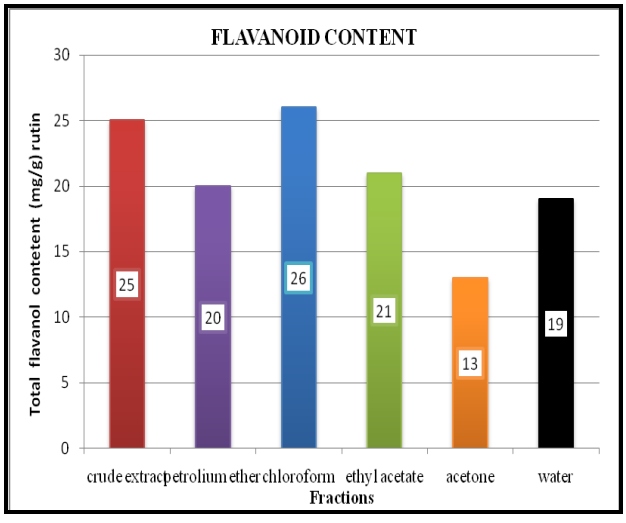

Figure 2: Estimation of Flavanoid Content

The flavanoid content of other fractions were observed to be 20±2.864, 21±1.924, 13±236, 19±2.236 mg/g rutin for petroleum ether, ethyl acetate, acetone and water respectively.

The total flavanoid content of the crude extract and its fractions are represented in the figure.2.

Evaluation of Antioxidant Activity

In vitro antioxidant activity was carried out to evaluate the antioxidant potential of the *acorus calamus* hydro ethanolic extract and its fractions by standard methods.

Diphenyl Picryl Hydrazyl (DPPH) Radical Scavenging Method

In The Present Study The Radical Scavenging Activity Was Carried Out For The Crude *Acorus Calamus* Extract and its Fractions. The Crude Extract and all its fractions have shown radical scavenging property. Among the crude extract and all its fractions**.** Chloroform and

ethyl acetate fractions have shown relatively good antioxidant potential with an IC_{50} value of 40±2.074 µg/ml and 30±1.949 µg/ml. The other four fractions also showed radical scavenging property with IC50 values of 62±3.175, 98±3.564, and 120±2.40 µg/ml. The IC_{50} values for all the fractions and the crude extract is shown in the figure.3.

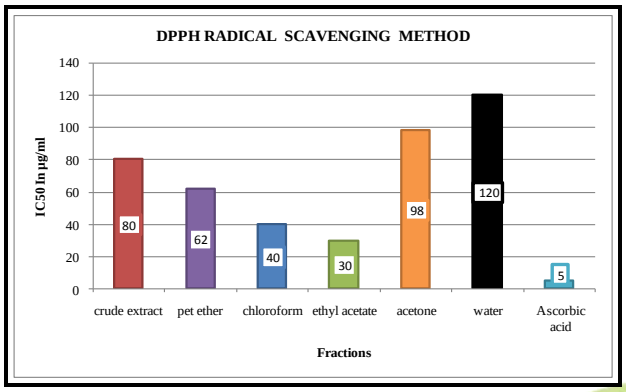

Figure 3: DPPH Radical Scavenging Activity

ABTS (2,2' azinobis- (3- ethylbenzothiazoline-6-sulfonicacid) Radical Scavenging Method

This method determines the radical scavenging activity of the extract and its fractions. Among all the fractions and the crude extract, ethyl acetate fraction showed good antioxidant property with an IC_{50} value of 5±2.75µg/ml. Other fractions also showed relatively good antioxidant activity and the IC_{50} values are presented in the figure 4.

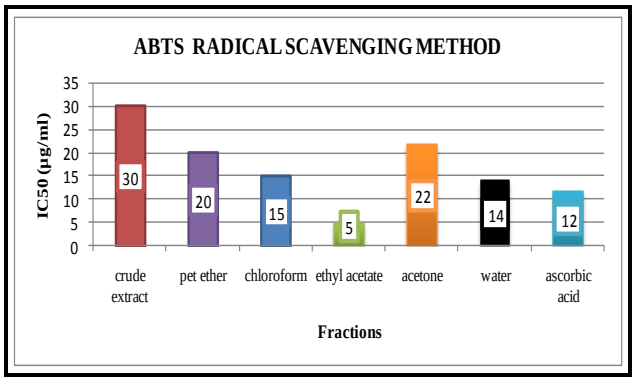

Figure 4: ABTS Radical Scavenging Method

Scavenging of Superoxide by Alkaline DMSO Method

Superoxide anion radical scavenging activities of crude methanolic extracts of *Acorus calamus* and its fractions were estimated. Among the crude extract and its fractions the ethyl acetate fraction showed better antioxidant activity with an IC_{50} value of 32±3.495 µg/ml. Other fractions also showed considerable antioxidant activity which is presented in figure 5.

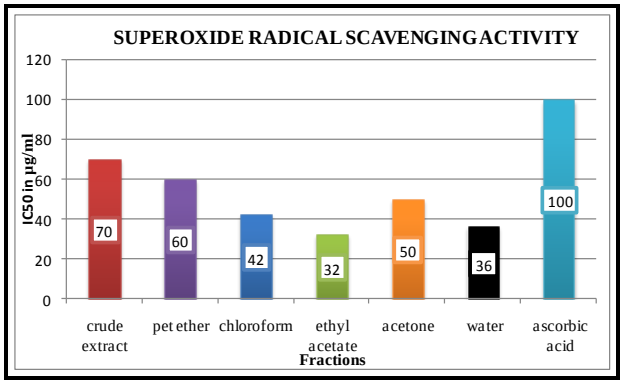

Figure 5: Superoxide Radical Scavenging Method

Scavenging of Hydrogen Peroxide Radicals

Hydrogen peroxide radical scavenging activity of the crude extract and its fractions are estimated. Among the crude extract and its fractions only the ethyl acetate fraction showed relatively good antioxidant activity with an IC_{50} value of 88±3.647µg/ml. all other fractions and the crude extract showed only a moderate antioxidant activity. The results are shown in the figure 6.

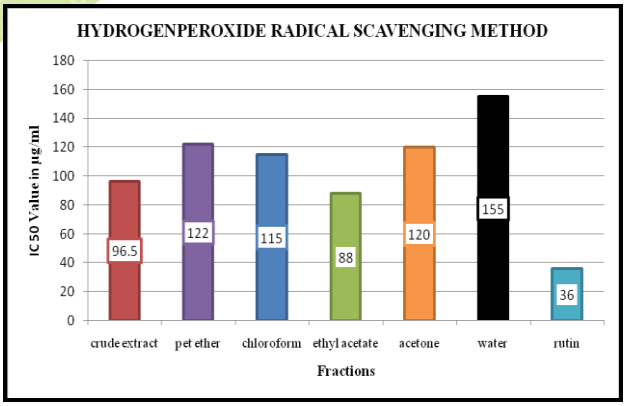

Figure 6: Hydrogen Peroxide Radical Scavenging Method

Estimation of Total Antioxidant Capacity by Phosphomolybdenum Method

The estimation of total antioxidant capacity of the crude extract and its fractions was carried out. Among the crude extract and its fractions

ethyl acetate fraction showed the maximum antioxidant activity with 0.14±.029 mM equivalent of ascorbic acid. The other extracts also showed comparatively good antioxidant activity and the results are presented in the figure 7.

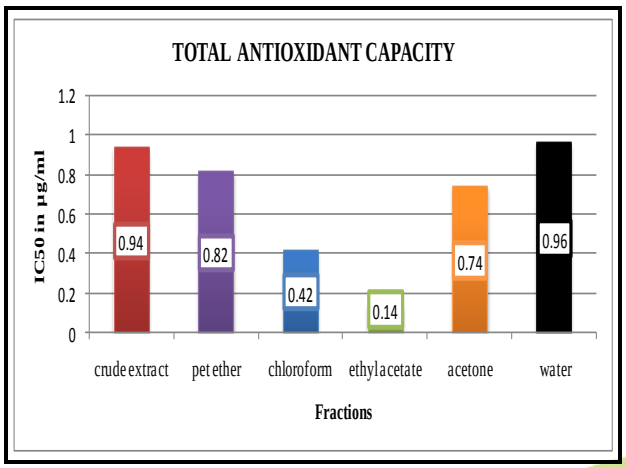

Figure 7: Total Antioxidant Capacity

Evaluation of Antimicrobial Activity

Antibacterial and antifungal activity of the *Acorus calamus* crude extract and its fractions; petroleum ether, chloroform, ethyl acetate, acetone and water were performed by cup plate method and minimum inhibitory concentration method.

Among the hydro ethanolic crude extract of *Acorus calamus* and its fractions, mainly the chloroform and ethyl acetate fractions showed relatively good antibacterial potential at a concentration below 500µg/ml against both gram positive and gram negative microorganisms, by cup plate method, the zone of inhibition ranging from 13 to 25mm diameter. The chloroform fraction showed a moderate antifungal activity at a concentration of 500µg/ml, by cup plate method, the zone of inhibition was found to be 12 to 19 mm diameter. The results were shown in the table 3 and 4.

Table 3: Antimicrobial studies of the crude extract and its fractions by cup plate method

Microorganism	Zone of inhibition in mm (All concentrations used were in 500µg/ml)						
	Crude extract	Pet ether	Chloroform	Ethyl acetate	Acetone	Water	Standard
Gram positive							
Mycobacterium phlei	16	12	15	22	12	11	17
B. subtilis	12	15	16	21	12	17	18
S.aureus	11	9	18	23	10	13	17
B. cereus	-	6	13	18	11	14	16
Gram negative							
E. coli	-	6	13	13	5	10	16
P. auregenosa	5	11	12	14	10	9	15
K. pneumonia	10	8	12	13	7	10	11
Fungi							
A.niger	-	-	12	13	9	11	21
C. albicans	-	4	13	9	12	8	22
S cervaceae	6	9	19	13	12	14	24

Table 4: Determination of Minimum Inhibitory Concentration of the crude extract of *Acorus calamus* and its fractions

Microorganism	Minimum Inhibitory Concentration (µg/ml)						
	Crude extract	Pet ether	Chloroform	Ethyl acetate	Acetone	Water	Standard
Gram positive							
Mycobacterium phlei	>1000	>1000	>1000	500	500	>1000	7.5
B. subtilis	>1000	>1000	>1000	500	500	>1000	9
S.aureus	>1000	>1000	>1000	500	500	>1000	12.3
B. cereus	>1000	>1000	**>1000**	500	500	>1000	14.5
Gram negative							
E.coli	>1000	>1000	>1000	500	500	>1000	20.1
P. auregenosa	>1000	>1000	>1000	500	>1000	>1000	15.4
K. pneumonia	>1000	>1000	>1000	500	>1000	>1000	16.3
Fungi							
Aspergillus niger	>1000	>1000	>1000	500	>1000	>1000	23.3
Candida albicans	>1000	>1000	>1000	500	>1000	>1000	18.3
Saccromyces cervaceae	>1000	>1000	>1000	500	>1000	>1000	23.5

In minimum inhibitory concentration method, ethyl acetate and acetone fractions showed relatively good antimicrobial activity at a concentration less than 500 µg/ml when compared to the crude extract and the other fractions.

Cytotoxicity Studies for the *Acorus calamus* Fractions

Determination of Cytotoxicity in MCF-7 Cell line Using SRB Assay

Cytotoxicity studies for the *Acorus calamus* fractions were carried out on MCF-7 cell culture. Concentrations 500-15.625µg/ml was assayed for each fraction. The cytotoxicity was measured as the growth inhibition and determined by SRB assay.

Among the five fractions of *Acorus calamus*, chloroform fraction showed reasonably good cytotoxicity against the MCF-7 cells with CTC_{50} value of 110 µg/ml, whereas the rest of the fractions were moderately cytotoxic to the MCF-7 cell line with CTC_{50} values ranging from 170- 360 µg/ml.

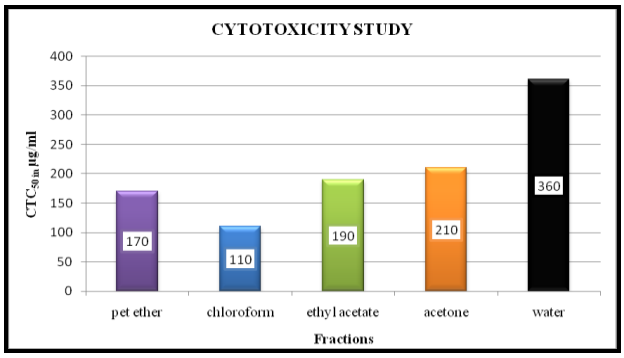

Figure 8: Cytotoxicity Study

DISCUSSION

Free radicals are chemical entities which can exist separately with one or more unpaired electrons. The propagation of free radicals can bring about thousands of reactions and may cause extensive tissue damage. Lipids, proteins and DNA all are susceptible to attack by free radicals. Antioxidants may offer resistance against oxidative stress by scavenging free radicals by their reducing ability.

DPPH (1,1 Diphenyl 2 picryl hydrazyl) is a stable nitrogen centered free radical which can be effectively scavenged by antioxidants and the absorbance can be taken at 517 nm. The reduction of DPPH by the process of either proton or electron donation which can be monitored spectrophotometrically, as DPPH upon reduction changes its color from violet to yellow. It is visually noticeable as a discoloration from violet to yellow. The extent of DPPH radical scavenged was determined by the decrease in intensity of violet colour in the form of IC_{50} value. Therefore substances capable of reducing DPPH can be considered as an antioxidant and a radical scavenger. Hence it is widely used for rapid evaluation of antioxidant activity of the plant extracts. Superoxide anions are relatively weak oxidants that produce very strong and harmful hydroxyl radicals and singlet oxygen can which are active agents of the oxidative stress.

Phenolics and flavanoids possess many useful biological properties such as anti-inflammatory, antimicrobial, enzyme inhibition, antiulcer, antioxidant etc. their antioxidant activities is because of the hydroxyl group (-OH) in the aromatic ring, which mediates redox reactions and is capable of scavenging free radicals.

In the present study, marked variations were observed in DPPH scavenging activity, ABTS radical scavenging activity, superoxide scavenging activity, hydrogen peroxide radical scavenging activity and total antioxidant capacity of each *Acorus calamus* fraction was highly correlated with the phenolic and flavanoid content of the extract. The ethyl acetate and chloroform fractions were observed to have maximum amount of phenolic and flavonoid content and showed good antioxidant property.

Antimicrobial study also reveals the antimicrobial property of the *Acorus calamus* chloroform and ethyl acetate fractions.

The cytotoxic study for *Acorus calamus* fractions revealed that the chloroform fraction showed the moderate toxicity, isolation of the pure compound which could be developed as a precursor for anticancer drugs.

CONCLUSION

Acorus calamus showed very promising antioxidant and antimicrobial activity in its ethyl acetate and chloroform fractions which are highly correlated with the phenolic and flavanoid content of the fractions. The chloroform fraction may be considered as a potential source of metabolites which could be developed as a precursor for anticancer drugs.

Further studies using *in vivo* models using these fractions may prove its significance in many disease conditions. Isolation of active constituents from these fractions may lead to the invention of new lead molecule for the treatment of many diseases.

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