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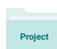
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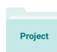
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DETERMINATION OF BIOLOGICAL PROPERTIES OF *Alocasia Macrorrhizos*: A MEDICINAL PLANT

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ABSTRACT

The present study was designed and conducted to detect possible phytochemicals, and investigate antioxidant, antimicrobial, thrombolytic, cytotoxic and anthelmintic activity of the methanolic extract of *Alocasia macrorrhizos* and its different fractions. Phytochemical screening was carried out using the standard test methods of different chemical groups. For assessing the antioxidant activity, DPPH free radical scavenging assay was carried out. For the evaluation of in vitro antimicrobial activity, disc diffusion method was used. The method of Prasad et al., 2007 with minor modifications was used to determine the thrombolytic activity. Evaluation of cytotoxic activity was done using the brine shrimp lethality bioassay. And the other study was undertaken to evaluate anthelmintic activity (using *Pheretima posthuma* model) where piperazine was used as reference standard. The methanolic extracts were a rich source of phytochemicals. It contains moderate DPPH scavenging activity although methanolic extracts showed highest free radical scavenging activity (IC_{50} value is 47.11 μg/ml) while compared to that of the reference standards ascorbic acid . Morever, the methanolic and carbontetrachloride fraction of extracts revealed good antimicrobial activity at the concentration of 400 μg/disc and petroleum ether fraction and aquous fraction of crude extract shows good antifungal activity. By comparing

with the negative control, the mean clot lysis % was significant (p value <0.0009). The methanolic crude extracts also possess cytotoxic principles and have moderate cytotoxic potency. Comparing the value of plant extracts to the standard reveals the Methanolic extract of *Alocasia macrorrhizos* also shows good anthelmintic activity Therefore, further studies are suggested to determine the active compounds responsible for the biological activities of the plant extracts.

KEYWORDS: Phytochemical, antioxidant, antimicrobial, thrombolytic, anthelmintic, cytotoxic.

INTRODUCTION

Drugs, which are acquired from natural sources, play an important role in the treatment and prevention of human diseases. Traditional medicine is still one of the primary healthcare systems in many developing countries [1, 2]. Herbs are widely utilized in the traditional medicine and their remedial potentials are well established [3] .There are about 61% of new successful drugs developed between 1981 and 2002 were founded on natural products. Especially in the areas of cancer and infectious disease.[4] Recent propensity, however, exhibit that the discovery rate of active novel chemical entities is declining [5]. Natural products of different plants may give a new source of antimicrobial, antioxidants, antidiarrheal, anti-inflammatory and thrombolytic agents with possibly novel mechanisms of action [6, 7]. Bangladesh possesses a rich flora of Medicinal plant. Out of the estimated 5000 species of phanerogams and pteridophytes growing in this country more than a thousand are regarded as having medicinal properties [8]. Much work has been done on ethnomedicinal plants in Bangladesh. A large number of plants included in Araceae family have been investigated all over the world. Araceae plants, which are investigated in Bangladesh shows wide range of secondary metabolites including anti-diabetic, anti-diarrhoeal, antioxidants, thrombolytic and anti-inflammatory activity. Plants of the species *Alocasia,* under the family Araceae, have been reported having antimicrobial activity (Alocasia indica) [9] antioxidant activity (Alocasia fornicata) [10]. In this research an attempt has been taken to study the pharmacological activities of another member of Alocasia species; *Alocasia macrorrhizos,* member of the Araceae family, which is adequately growing in Bangladesh. *Alocasia macrorrhizos* (*A. macrorrhizos*) is a robust herb with long, cylindrical caudex and large leaves. The rootstock is cooling, diuretic and mild laxative. It is useful in piles, constipation, inflammations, rheumatism, leprosy, jaundice, diseases of spleen. The ash of the rootstocks

mixed with honey is used in cases of aphthae. The leaf Juice is localy used as astringent. In Chittagong Hill Tracs the rootstock is extensively used in diarrhoea and stomachache [11]. But there are insufficient records in literature of this plant, regarding its pharmacological activities and physicochemical characteristics. Plants contain various phytochemicals such as Carbohydrates, Alkaloids, Saponins, Phytosterols, Phenol, Tannins, Flavonoids, and Proteins and amino acids. These phytochemicals have various pharmacological activities like thrombolytic activity [12], antimicrobial activity [13], antioxidant activity [14]. Antibiotics are one of the most important weapons to eliminate bacterial infections and have greatly enriched the quality of human life since their indication. However, over the past few years, these health advantages are now under threat as many generally used antibiotics have become less effective against certain diseases. Not only because many of them produce toxic response, but also due to dominance of drug-resistant bacteria. It is indispensable to investigate newer drugs with lower chance of resistance [15].

Atherothrombotic diseases such as myocardial or cerebral infarction are severe consequences of the thrombus formed in blood vessels [16, 17]. Streptokinase (SK), Tissue plasminogen activator, urokinase, etc. are used as thrombolytic agents to dissolve the clots in the blood vessels [18, 19]. However, these drugs have particular limitations which cause severe and sometimes fatal disorders such as hemorrhage, lacked specificity, severe anaphylactic reaction etc. Moreover, because of immunogenicity, multiple treatments with SK for a specified patient are limited [20]. Agents from plant origin are likely to be less antigenic and inexpensive [21]. Significant efforts should made for finding and progress of natural products from different plants and animal sources which have anticoagulant [22], antiplatelet [23], antithrombotic and thrombolytic activities [12]. Antioxidants scavenge free radicals and also reduce oxidative damage, thus decreasing the risk of disease induced by free radicals [24]. A proper balance between oxidation and antioxidation is believed to be critical for sustaining healthy biological system. Synthetic antioxidants may have adverse effects on human body; hence, much attention has been put toward natural antioxidants. And plants are nice sources of natural antioxidants [25]. Currently available anti-tumor drugs: nitrogen mustards, mercaptopurine, carboplatin, azathioprine have been associated with serious side effects. If any significant cytotoxic effect exerting herbal medicine can be obtained which is locally available and relatively cheap then it will be very helpful in the treatment of cancer [34]. Brine shrimp lethality Bioassay developed by Mayer et al. (1982) is widely used as a simple, reliable and cheaper prescreens method to select bioactive compounds, especially antitumor

compounds from the natural source [35] . Parasitoses have been of concern to the medical field for centuries and the helminthes still cause considerable problems for humans and animals. Now a day the most of the medicinal preparations available in the market are either not fruitful up to the mark or has exhibited resistance resulting in reoccurrence again. Drugs from plant origin serve as prototype to develop more effective and less toxic medicines [36]. Following these hypothesis, the present study focuses on screening of methanolic extract of *A. macrorrhizos* for the study of antioxidant activity, antimicrobial activity, thrombolytic activity, cytotoxic activity and anthelmintic activity by using an in vitro and in vivo assay method.

MATERIALAND METHOD

Plant Materials

For this present investigation, the *A. macrorrhizos* were collected from Chittagong in December, 2011. For the taxonomic identification of the plant was carried out with the help of taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh (Accession no. 38303). The voucher specimen was also deposited there for future reference.

Chemicals and Reference Drug

For performing these experiments , Trichloro acetic acid (TCA), 1,1-Diphenyl-2-picryl hydrazyl (DPPH), L- Ascorbic acid, Folin-ciocalteu phenol reagent, Butylated Hydroxy Anisole (BHA), Gallic acid, phosphate buffer (pH 6.6), distilled water, streptokinase (30000 IU and 15000 IU) of analytical grade (Merck, Darmstadt, Germany) were used. All the reagents and chemicals were purchased from E. Merck (Germany) and Sigma Chemical Co. Ltd, (St. Louis, MO, USA).

Extraction of Plant Materials

For methanolic extraction 400 gm of air dried and powdered sample were submerged in 2500 ml of 80% methanol (Merck KGaA, Darmstadt, Germany) in clean, sterilized and flat-bottomed glass container. The container and its contents was sealed and kept for maceration for 20 days with occasional shaking and stirring. At the end of 20th day, the whole mixture was filtered using filter cloth and Whatman® filter paper (Sargent-Welch, USA). The resultant filtrate was then allowed to evaporate in water bath maintained 45°C to dryness and thus a greenish black colored semisolid extract was obtained (yield 25 gms). This gummy concentrate was denominated as crude extract of methanol.

Solvent-Solvent Partitioning

Solvent-solvent partitioning was done using slightly modified protocol designed by Kupchan [34]. The methanolic extract (5 gm) was dissolved in 10% aqueous methanol. It was extracted firstly with Petroleum Ether, then with carbon tetrachloride and then finally with Chloroform.

Phytochemical Screening

Small quantity of methanolic extract and different fractions of *A. macrorrhizos* were subjected to preliminary phytochemical screening for the detection of phytochemicals such as alkaloids with Mayer's and Hager's reagent, Benedict's test and Fehling's test for Carbohydrates, glycosides with Legal's test and Borntrager's test, Salkowski's test and Libermann Burchard's test for phytosterols, proteins with xanthoproteic test, alkaline reagent test and lead acetate test for flavonoids, tannins with gelatin test, Froth test and foam test for saponins, phenols with ferric chloride test [21].

Antioxidant Activity

There are different well known methods to determine the antioxidant properties. Among them, two complementary test methods namely DPPH free radical scavenging assay method and total phenolic content determination were used for investigating the antioxidant activity of *A. macrorrhizos*

Total Phenolic Content Determination

The amount of total phenolics in extracts was determined with the Folin-Ciocalteu reagent [21]. Here, gallic acid was used as a standard and the amount of total phenolics were expressed as mg/g of gallic acid equivalents (GAE). Concentration of 6.25, 12.5, 25, 50, and 100 µg/ml of gallic acid and concentration of 2 µg/ml of plant extract were prepared in methanol and 0.5 ml of each sample were placed into test tubes and mixed carefully with 2.5 ml of a 10- fold dilute Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The test tubes were covered with para-film and allowed to stand for almost 30 min at room temperature before the absorbance was read at 760 nm spectrophotometrically (UV-1800, Shimadzu, Japan). All determinations were performed nicely in triplicate [21]. Thus, total phenolic content was determined as mg of gallic acid equivalent per gram using the equation obtained from a standard gallic acid calibration curve.

DPPH Scavenging Activity

DPPH radical serves as the oxidizing radical to be reduced by the antioxidant (AH) and as the indicator for the reaction. The stable DPPH radical-scavenging activity was measured using the modified method described by Gupta [17]. In this assay, 2 ml of 0.2 mµ methanolic DPPH solutions was added to 2 ml of extract solution at different concentrations and the contents were stirred vigorously for 15 seconds. Then the solutions were allowed to stand at dark place at room temperature for 30 min for reaction to occur. After 30 min, absorbance was measured against a blank at 517 nm with a double beam UV/Visible spectrophotometer. The percentage of DPPH radical-scavenging activity of each plant extract was calculated as DPPH radical-scavenging activity (%I),

$$= \frac{A_0 - A}{A_0} \times 100$$

Where,

A0 is the absorbance of the control solution (containing all reagents except plant extracts);

A is the absorbance of the DPPH solution containing plant extract.

The DPPH radical-scavenging activity (%) was plotted against the plant extract concentration to determine the concentration of extract necessary to decrease DPPH radical-scavenging by 50% (called IC50). The IC50 value of each extract was estimated by sigmoid non-linear regression. These values were changed to antiradical activity, defined as 1/EC50, since this parameter increases with antioxidant activity. All determinations were performed in triplicate.

Antimicrobial Activity

Two strains of Gram-positive (*Staphylococcus aureus, Bacillus subtilis*), three strains of Gram-negative bacteria (*Pseudomonas aeruginosa, Escherichia coli, Salmonella typhi*), and two strains of fungi (*Candida albicans, Aspergillus niger*) were used to assess the antimicrobial activity. The organisms were properly sub cultured in nutrient broth and nutrient agar. They were collected from the, Noakhali Science & Technology University (Department of Microbiology), Bangladesh. Disc diffusion method is widely acceptable for the evaluation of antimicrobial activity [27]. In this classical method, antibiotics were diffused from a reliable source through the nutrient agar and a concentration gradient was created. Dried, sterilized filter paper discs (6 mm diameter) containing the test samples of known amounts (400 µg/disc) were placed on nutrient agar medium consistently seeded with the test

microorganisms. Standard antibiotic, Ciprofloxacin (5 µg/disc) was used as positive and blank discs were used as negative control. For the highest diffusion of the test materials to the surrounding media, these plates were kept at low temperature (4 °C) for 24 h**.** Then the plates were incubated at 37 °C for about 24 h to allow optimum growth of the organisms. The test samples having antimicrobial property inhibited microbial growth in plates and thereby yielded a clear and distinct zone, which is defined as zone of inhibition. The antimicrobial activity of the test materials was then ascertained by measuring the zone of inhibition denoted in millimeter.

Thrombolytic Activity

In vitro clot lysis activity of *A. macrorrhizos* was carried out following to the method of Prasad et al., 2007 [18] with minor modifications.

Streptokinase (SK)

To the commercially available lyophilized S-Kinase™ (Streptokinase) vial (Batch no: VEH 14, Popular Pharmaceuticals Ltd., Bangladesh) of 15, 00,000 I.U., 5 ml 0.9% sodium chloride (NaCl) was added and properly mixed. Then the solution was diluted up to 300000IU and 15000IU conc. This solution was used as the reference standard for thrombolytic activity [18] .

Specimen

Venous blood (5 ml) was drawn from healthy human volunteers (n =10) who do not have a history of oral contraceptive or anticoagulant therapy (using a protocol approved by Institutional Ethics Committee). A consent form was filled up for every volunteer before collecting their blood sample. 500 µl of blood was transferred to each of the formerly weighed micro centrifuge tubes to form clots [18].

Preparation of Sample

The prepared NaCl solution (0.9%) was used to make various concentrations of plant extract: 5, 10 and 20 mg/Ml.

Study Design

Venous blood drawn from healthy volunteers (n = 10) was instantly citrated using sodium citrate solution (3.1%) and then was shifted in several pre-weighed sterile micro centrifuge tube (500 µl/tube). 200 µl of 2% calcium chloride was then added to each of these tubes, mixed well and incubated carefully at 37°C for 45 minutes for clotting to occur. After clot

formation, serum was completely removed (aspirated out without disturbing the clot formed) and each tube having clot was again weighed consciously to determine the clot weight (clot weight = weight of clot containing tube – weight of each tube). Each micro centrifuge tube containing clot was properly labeled and five hundred microlitre of different concentrations of the plant extract such as 5 mg/mL (n = 10), 10 mg/mL (n = 10) and 20 mg/mL (n = 10) or saline (negative control) (n =10) or 30,000 I.U. or 15000IU reference drug (n = 10)] was added to tubes with clots. All the tubes were incubated at 37ºC for almost 90 min. The supernatant fluid was then carefully removed and the tubes were properly weighed again. The weight difference before and after clot lysis was expressed as % clot lysis [18]. The result was expressed as percentage of clot lysis following the underneath equation.

% of clot lysis = (wt. of released clot /clot wt.) × 100

Statistical Analysis

The results are expressed as mean ± SEM. Statistical comparisons were made using one-way ANOVA with Dunnett t test. Significance was set at $p < 0.05$. Dose dependencies were determined by the regression coefficient (r).

Evaluation of Cytotoxicity

There are sufficient experimental evidences reporting the use of brine shrimp for environmental studies [29], screening for natural toxins [30] and as a general screening for bioactive substances in plant extracts. For the study purpose, *Artemia salina* as a test object and a developed protocol was used in Brine shrimp lethality bioassay to monitor cytotoxicity of *A. macrorrhizos* [31]. Brine shrimp eggs were hatched in simulated seawater to get nauplii. Sample solutions were prepared by dissolving the test materials in pre-calculated amount of dimethyl sulfoxide (DMSO). Ten nauplii were taken in vials containing 5 ml of simulated sea water. The samples which are in different concentrations were added to the pre-marked vials with the help of micropipette. The assay was accomplished using three replicates. Survivors were counted after 24 hours. 38 g sea salt (pure NaCl) was weighed, then dissolved in one liter of distilled water and filtered off to get clear solution. *A. salina* leach (brine shrimp eggs) collected from pet shops was used as the test organism. A small tank is used to hold saline water and shrimp eggs were added to the tank. Two days were allowed to hatch the shrimp and developed to be nauplii. Constant oxygen supply was provided throughout the hatching time. A Pasteur pipette was used to add 10 live shrimps to each of the vials containing 5 ml of seawater. Clean test tubes were used for ten different concentrations (one test tube for each

concentration) of test samples and ten test tubes were taken for standard drug Vincristine sulphate for ten concentrations of it and another one test tube for control test. Then 100 µl of solution was taken in test tube each containing 5 ml of simulated seawater and 10 shrimp nauplii. Thus, ultimate concentration of the prepared solution in the first test tube was 400 µg/ml and others are 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0.781 g/ml. Vincristine sulphate (10, 5, 2.5, 1.25, 0.625, 0.313, 0.156, 0.0781 and 0.039g/ml) was used as positive control. A negative control group was also prepared containing sea water and 100 µl DMSO. After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

Evaluation of Anthelmintic Activity

In vitro anthelmintic activity can be screened using several worm samples like Ascaridia galli, Ascaris lumbricoides and *Pheretima posthuma*. But *P. posthuma* was most frequently used as test worm in anthelmintic activity determination, because it possesses anatomical and physiological similarity with the intestinal roundworm parasite of human [32]. The anthelmintic activity was performed according to the method of Ghosh *et al.* [33] on adult Bangladeshi earthworm *Pheretima posthuma*. The earthworms (*P. posthuma*) were collected from moist soil and washed with normal saline to remove all fecal matter were used for the anthelmintic study. According to the experimental protocol, earthworms of 3-5 cm in length and 0.1-0.2 cm in width were used. Various concentrations of each extract (Methanol and Water) were tested in the bioassay, which included determination of time of paralysis and time of death of the worms. Piperazine was used as standard reference and saline solution as control. Various weight of roots extract was weighed (100-500 mg). Then they were dissolved in 10 ml of distilled water in volumetric flask to prepare the solution concentrations of 10, 20, 30, 40 and 50 mg/ml gradually. 100 mg of standard reference piperazine was dissolved in 10 ml of distilled water. Now the concentration is 10 mg/ml. Earthworms were divided into four groups, each containing three worms in Petri dish. Then roots extract was applied to the Petri dish. And one is for reference.

RESULTS

Phytochemical Screening

The phytochemical screening of methanol extracts of *Alocasia macrorrhizos* showed varied results. The results are shown in Table-1.

S. No	Phytochemicals	Test	Result
1	Alkaloids	Wagner's test	+
		Hager's test	+
2.	Carbohydrates	Hager's test	+
		Benedict's test	+
		Fehling's test	+
3	Glycosides	Legal's test	-
4	Saponin	Froth Test	+
5	Phytosterols	Libermann-Burchard's test	+
6	Phenol	Ferric Chloride Test	+
7	Tannins	Gelatin Test	+
8	Flavonoids	Alkaline reagent test	+
		Lead acetate test	+
9	Proteins and amino acids	Xanthoproteic Test	+
		Ninhydrin Test	+
10	Terpenes	Copper acetate Test	-

Key: (+) = Present and (−) = Absent

The phytochemical analysis conducted on of *A. macrorrhizos* extract revealed the presence of Alkaloids, Carbohydrates, Saponin, Phytosterols, Phenols, Tannins, Flavonoids, Proteins, and Terpenes on crude extracts. The presence of Alkaloids, Phytosterols, Phenols, Tannins, and Flavonoids are known to be useful as antimicrobial agent [28]. Saponin shows thrombolytic activity [29]. The presence of phenolic compounds and flavonoids in this plant contributed to their antioxidative properties and thus the usefulness of these plants in herbal medicament [21].

DPPH Scavenging Activity

DPPH free radical scavenging activity of crude extracts of *A. macrorrhizos and* their different soluble fractions were found to be increased with the increase of concentration of the extract (Table 3). Different partitions of methanolic extract of *A. macrorrhizos* were subjected to free radical scavenging activity. Here, Ascorbic acid was used as reference standard. In this investigation, the methanolic crude extract showed the highest free radical scavenging activity with IC50 value 47.11µg/ml. At the same time the petroleum ether soluble fractions, Carbon tetrachloride, aqueous and chloroform fractions also exhibited moderate antioxidant potential having IC50 value 65.04µg/ml, 107.34µg/ml, 170.13µg/ml, 201.39µg/ml respectively.

Table-3: Comparative DPPH radical scavenging activity of different extracts of *Alocasia macrorrhizos* standards of Ascorbic Acid (AA).

% Inhibition of different solvent extract and Standard						
Concentration (µg/ml)	**Methanolic Crude Extract**	**Carbon Tetra Chloride Extract**	**Petroleum Ether Extract**	**Chloroform Extract**	**Aquous Extract**	**Ascorbic Acid (Standard)**
500	78.81	63.44	74.92	61.93	60.55	97.40
250	76.72	58.61	71.60	52.57	53.97	83.96
125	72.84	54.08	65.56	47.13	48.22	72.29
62.5	64.48	44.11	52.27	36.56	41.92	65.44
31.25	49.85	39.88	44.71	27.19	36.44	59.03
15.625	22.09	31.42	22.96	18.73	20	56.85
7.813	13.73	21.45	9.97	14.80	15.34	54.29
3.90	7.76	11.18	6.34	9.06	12.60	49.46
1.953	5.07	6.65	2.72	5.74	11.78	41.66
IC50 (µg/ml)	47.11	107.34	65.04	201.39	170.13	14.52

Antimicrobial Activity

The antimicrobial potency of the test agents are measured by their activity to prevent the growth of the microorganisms (at concentrations of 400 µg/disc.) surrounding the discs which gives clear zone of inhibition. After incubation, the Antimicrobial activities of the test materials such as Methanolic crude extract (MAM), Pet. Ether fraction of crude extract (PEAM), Chloroform fraction of crude extract (CAM), Carbon tetra Chloride fraction of crude extract (CTCAM) and Aqueous fraction of crude extract (AAM) were determined by measuring the diameter of the zones of inhibition in millimeter with a transparent scale (Table 4). Here, Standard antibiotic disc of ciprofloxacin (5µg/disc) was used for the comparison.

Table-4: Results Of in Vitro Antimicrobial Screening of *Alocasia macrorrhizos*.

Types of micro organism		**Samples of *A. macrorrhizos* - zone of inhibition mm**					**Ciprofloxacin**
		PEAM	MAM	CTCAM	CAM	AAM	
Gram Positive Bacteria	*Bacillus subtilis*	8.8	11.1	8.8	Nil	9.7	41.7
	Staphylococcus aureus	Nil	14.1	9.9	Nil	11.6	32.7
Gram Negative Bacteria	*Pseudomonas aeruginosa*	Nil	8.6	8.7	8.4	9.4	41.8
	Salmonella typhi	8.8	9.1	11.4	11.7	Nil	36.7
	Escherichia coli	11.7	7.5	11.4	8.4	9.4	32.8
Fungi	*Candida albicans*	11.9	9.5	Nil	Nil	8.9	34.8
	Aspergillus niger	10.7	7.7	Nil	Nil	7.7	45.4

In case of *A. macrorrhizos* samples, several samples were found to be fairly working against some gram positive and negative bacteria and fungi. The zones of inhibition produced by

crude methanolic extract were ranged from 7.5-14.1 mm, while the Pet. Ether soluble fraction of the methanolic extract showed 8.8-11.9 mm, carbon tetra chloride soluble fractions showed 8.7-11.4 mm, aqueous fractions showed 8.9-11.6 mm and Chloroform soluble fractions showed 8.4- 11.4 mm at a concentration of 400 g/disc. The Chloroform soluble fractions of the methanolic extract exhibited inhibitory activity against selectively on gram negative bacteria, having no effect on gram positive bacteria and fungi. There are fractions of the plant found not be exerting any effect against Gram positive (CAM), negative bacteria (PEAM and AAM) and Fungi (CTCAM, CAM). Considering the above results, it can be said that there are compounds responsible for antimicrobial activities against different gram positive and negative bacteria especially in MAM and CTCAM fractions and compounds responsible for antifungal activities against different fungi especially in PEAM, MAM and AAM fractions. Therefore, further investigation can be recommended to detect chemical compounds exerting antimicrobial and antifungal effects of these fractions.

Thrombolytic Potential

Streptokinase (30000 and 15000 I.U.) as a positive control to the clots along with 90 minutes of incubation at 37 degree centigrade, showed 45% and 26% clot lysis respectively. Clots when treated with 100 microliters 0.9% saline water (negative control) showed only 5.5% blood clot lysis. The mean difference in clot lysis percentage between positive and negative control was very significant (p value <0.0009). By comparing with the negative control the mean clot lysis percentages of methanolic and petroleum ether extract of *A. macrorrhizos* was significant (p value <0.0009).

Table-5: Results Of % of clot lysis of crude methanolic extract and petroleum ether fraction of *A. macrorrhizos,* Streptokinase and 0.9% Nacl.

Sample name	Concentration	% of clot lysis
Methanolic crude extract	5	10.794
	10	7.82
	20	6.35
Petroleum ether fraction	5	9.12
	10	7.98
	20	6.92
Streptokinase as standard	15000 I.U.	26.14
	30000 I.U.	45.35
0.9% saline water as Control		5.57

Cytotoxicity Test

The LC50 values of crude methanol extract, petroleum ether, chloroform, carbon tetra chloride and aqueous extract of *A. macrorrhizos* found to be 0.192, 7.937, 12.437, 21.65 and 0.331 g/ml, respectively (Table 7). The positive control vincristine sulphate showed LC50 at a concentration of 0.544 µg/ml.

S. No	Sample Name	Regression Line	R^2	$LC_{50\ value}$
1	Vincristine (Positive Control)	y = 33.22x + 58.78	$R^2 = 0.958$	0.544
2	Alocasia macrorrhizos Methanolic extract	y = 17.31x + 62.40	$R^2 = 0.918$	0.192
3	Alocasia macrorrhizos Petroleum Ether	y = 31.60x + 21.57	$R^2 = 0.971$	7.9374
4	Alocasia macrorrhizos Carbon tetra Chloride	y = 28.38x + 29.58	$R^2 = 0.963$	5.2422
5	Alocasia macrorrhizos Chloroform fraction	y = 38.85x - 7.470	$R^2 = 0.965$	12.4372
6	Alocasia macrorrhizos aqueous fraction	y = 35.23x - 2.95	$R^2 = 0.957$	21.65

Table 6: Summary of the Cytotoxicity In Brine Shrimp Bioassay.

From the results of the brine shrimp lethality bioassay of *A. macrorrhizos* extracts it can be well predicted that the all form of extracts possess cytotoxic principles except chloroform.

Anthelmintic Test

The crude methanolic extracts of *A. macrorrhizos* produced a significant anthelmintic activity in dose dependent manner and the activity of methanol extract was comparable with that of standard drugs, which is shown in table 7.

Table 7: Time taken for paralysis and death of earthworms for methanol extract of *Alocasia macrorrhizos*, standard drug and control.

Test substance	Concentration (mg/ml)	Time taken for paralysis (min)	Time taken for death (min)
Control (Distilled water)	0	-	-
Standard (Piperazine)	10	14.75 ± 0.408	23 ± 1.443
Methanol extract of *Alocasia macrorrhizos*	10	84.25 ± 2.056	94.25 ± 2.496
	20	60.5 ± 1.323	69.5 ± 1.658
	40	55.5 ± 3.175	65.75 ± 2.720
	60	45.5 ± 2.062	56.25 ± 1.548
	80	45 ± 1.826	51.5 ± 1.848

It is observed that *A. macrorrhizos* paralyzed the entire worm within an average 45 min and killed all the worms within 51 min. In both cases the concentration was highest (80 mg/ml)

and paralysis and death occurring time increased with the decrement of concentration. The average effect of the standard 10mg/ ml piperazine was 14.75 for paralysis and 23 min for death. And the negative blank control did not any efficacy either in paralyzing or death.

DISCUSSION

Medicinal plants containing phytochemicals are well known because they show a variety of pharmacological actions in human body [20] and in our study preliminary phytochemical screening showed the presence of various phytochemicals. The presence of polyphenolic compounds such as flavonoids and tannins are responsible for antioxidant activity of the extracts [21]. In this investigation the extract of the plant showed moderate antioxidant activities with an IC50 which were compared with the values of standard drugs used. Antioxidant activities of different extracts of *A. macrorrhizos* found to increase with the increasing concentration [22]. This plant is a good source of total phenolic content. It also contains moderate DPPH scavenging activity although carbon tetra chloride soluble fraction showed highest free radical scavenging activity. The effect of antioxidants of plant on DPPH is thought to be due to their hydrogen donating ability [22]. Therefore, the extracts of this plant could be used potentially for the prevention of free radical-mediated diseases. Since the present study showed the presence of various bioactive secondary metabolites such as flavonoids, tannins, saponin and alkaloids, that singly or in combination may be liable for the defense mechanism against microorganisms and insects [3]. For this reason, methanolic extract contains significant antimicrobial activity.

Platelets play an important role in the process of formation of thrombus on the endothelial surface [13]. Several thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels; but these drugs have some limitations which can lead to serious and sometimes fatal consequences [23]. In the present study, the plant extracts showed significant thrombolytic activity compared to negative control. This thrombolytic activity may be due to the fact that the extracts are good sources of flavonoids, alkaloids, terpenoids and tannins which are said to exert blood clot lysis [23, 24]. From the results of the brine shrimp lethality bioassay, it can be well predicted that the methanolic crude extracts possess cytotoxic principles and have moderate cytotoxic potency. It was reported by several studies that our investigational plant *C. viscosum* possesses different types of phytoconstituents like carbohydrates, glycosides, sterols and triterpenoids, tannins, saponins, alkaloids, flavonoids etc. [18, 19]. So it may be assumed that the cytotoxic activity of the methanolic extract of *A.*

macrorrhizos may be due to the presence of these phytochemicals, although the exact compound responsible for the cytotoxic activity is yet to be discovered. Comparing the value of plant extracts to the standard we can say that the methanolic extract of *A. macrorrhizos* also shows good activity and this plant extract could be source of potent drugs acting against parasitic infections against human.

CONCLUSION

In the context of the above discussion, it can be revealed that the extracts of *A. macrorrhizos* showed nice antioxidant activity. These extracts also possess significant antimicrobial, clot lysis activity, moderate cytotoxic activity and good anthelmintic activity. However, further investigations based on these preliminary studies are required to explore the bioactive molecules which are responsible for the extracts' activities as well as their mechanisms of action.

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