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Original Article

Phytochemical screening, *in-vitro* antioxidant and cardioprotective potential of *monochoria vaginalis* on doxorubicin induced cardiotoxicity

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ABSTRACT

The present investigations were undertaken to identify photochemical present in the ethanolic leaf extract of Monochoria vaginalis by GC MS/MS, evaluation of the in vitro antioxidant and cardioprotective activity against DOX-induced cardiotoxicity in rats. The invitro antioxidant potential like DPPH radical scavenging activity, Reducing Power, Superoxide anion Scavenging activity and Nitric oxide Radical Scavenging Activity was assessed. The extent of heart damage and effect of the plant extract was assessed by various biochemical parameters like xanthine oxidase phospho lipase, phospho lipid, glycogen and lipid peroxidase were determined. In the *monochoria vaginalis* extract 16 compounds were identified by GC–MS/MS. Ethanolic extract of Monochoria vaginalis exhibit strong antioxidant activity. The pretreatment with *monochoria vaginalis* at two doses (250 mg/ kg and 500 mg /kg) to DOX treated rats significantly prevented the altered enzymes phospholipase, xanthine oxidase and lipid peroxides were enhanced following depletion of glycogen and phospholipids .This study showed that the antioxidant and cardioprotective potential of *monochoria vaginalis*, therefore scientifically embankment the use of this plant in traditional medicine for treatment of heart diseases.

Keywords: Cardioprotection, *Monochoria vaginalis*, Doxorubicin, xanthine oxidase, phospholipase, Antioxidant.

INTRODUCTION

A number of herbs are traditionally used in different countries during drug or toxin induced hepatic, renal and cardio disorders [1]. *Monochoria vaginalis* (MV) is a species of flowering plant is an aquatic plant which belongs to family Pontederiaceae, known by several common names, including carpet weed, heartleaf false pickerelweed, oval-leafed pondweed and Karunkuvalai in Tamil, and it is distributed throughout India.

The entire plant is eaten as a vegetable in India, and the roots are used medicinally. Its leaves are eaten as a pot herb and several parts of it are used as herbal medicine [2]. The leaf juice of *Monochoria vaginalis* is used to treat cough and that of roots is used to treat stomach & liver problems, asthma and tooth ache [3-4]. In Siddha system, the root is used to treat cough, disease of pittam, venereal disease, thirst, fainting and fever [3]. The aerial part of plant is considered as a functional food and has also been employed for the treatment of asthma and fever [5]. This plant is also resistant to several acetolactate synthase (ALS) inhibitors. In addition, it has been reported that n-butanol fraction of *Monochoria vaginalis* exhibited the highest antioxidant activity [6]. However no data are available on the cardioprotective and invitro antioxidant activities of MV. Therefore, this study was designed to investigate the phytoconstituents identification by GC–MS/MS and protective effects of the ethanol extract of MV against DOX-induced cardiotoxicity in rats.

MATERIALS AND METHODS

Plant material: Leaves of *Monochoria vaginalis* were collected, identified and authenticated by a Botanist, Dr.K.Shanthi, goverrtment arts college, Tiruvannamalai. Voucher specimen (KPCP-

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13/2016) was retained in the, PG Department of Pharmaceutical chemistry, Kamalakshi Pandurangan College of Pharmacy, Tiruvannamalai, Tamil Nadu, India.

Chemicals and Reagents: Doxorubicin was purchased from Microlabs, Tamilnadu. India. All other chemicals and solvent were of analytical grade and commercially available.

Acute toxicity studies: Acute oral toxicity study was performed as per OECD - 423 guidelines (acute toxic class method), Albino rats (n=6) of single sex were selected for the acute toxicity study. Which received a single oral dose of 2000mg/kg body weight of ethanol extract of *Monochoria vaginalis*. The dose was administered to overnight fasted rats and food was withheld for a further3-hours after administration of the drug and observed for signs of toxicity for a period of 14 days.

Extraction: Leaves (1000 g) of *monochoria vaginalis* were cleaned with water and dried, then they were powdered using a mechanical grinder to obtain a coarse powder. The coarse powder (500 g) was passed through 40 mesh sieve and extracted with ethanol (90/10 v/v) in a Soxhlet apparatus at 25°C. The extract was freeze-dried and stored in a vacuum desiccator and the yield was 7 g.

GC–MS/MS analyses of ethanol extract of *Monochoria vaginalis* for the determination of chemical composition

GC Programme: Column BR-5MS (5% Diphenyl / 95% Dimethyl poly siloxane), 30m x 0.25mm ID x 0.25µm df Equipment Scion 436-GC Bruker, Carrier gas 1ml per min, Split 10:1,Detector TQ Quadrupole Mass Spectrometer,Software MS Work Station 8,Sample injected 2µl.

Oven temperature Programme:- 110° C hold for 3.50 min,Up to 200° C at the rate of 10 ° C/min-No hold,Up to 280 ° C at the rate of 5° C / min- 9 min hold,Injector temperature 280° C,Total GC running time: 37.50 min,

MS Programme: Library used NIST Version-11,Inlet line temperature 290° C,Source temperature 250 ° C,Electron energy 70 eV,Mass scan (m/z) 50-500 amu,Solvent Delay 0 - 3.5 min,Total MS running time: 37.50 min.

The identification of the constituents of the ethanolic extract of *Monochoria vaginalis* was performed using a mass spectrometer (Agilent 6890/Hewlett–Packard 5975) fitted with an electron impact (EI) ion source. The ethanolic extract (2.0 µL) of *Monochoria vaginalis* was injected manually in the split mode with a Hamilton syringe to the GC–MS for total ion chromatographic analysis. For quantitative analysis, the selected ion monitoring (SIM) mode was employed.

Experimental animals: Studies were carried out using Wistar male albino rats (150–200 g), obtained from the Institute of Veterinary Preventive Medicine (IVPM), Ranipet, and Tamilnadu, India. The animals were housed in polyacrylic cages (38 cm, 23 cm, and 10 cm) and maintained under standard laboratory conditions with dark/light cycle (12/12 h). The animals were fed with standard pellet diet (supplied by the poultry research station, Nandhanam, India) and fresh water *ad libitum*. All the animals were acclimatized to lab conditions for a week before commencement of the experiment. All animal studies were performed in accordance to guidelines of CPCSEA and Institutional Animal Ethical Committee (IAEC) of Kamalakshi Pandurangan college of Pharmacy,Tiruvannamalai (Tamilnadu). CPCSEA registration number was 745/03/ac/CPCSEA and all the procedures were followed as per rules and regulation.

Induction of experimental myocardial infarction: Doxorubicin was dissolved in sterile double distilled water and injected subcutaneously in to rats (20 mg/kg) in group II, III and V respectively, after the last dose of the extract to induce Cardiotoxicity.

Experimental procedure

Group I. (Normal). Saline (0.75 ml/animal), orally for 14 days.

Group II. (drug control). Saline (0.75 ml/animal) + DOX 20 mg/kg, single intraperitoneal injection after 14th day.

Group III. *Monochoria vaginalis* (250 mg/kg), orally for 14 days + DOX (20 mg/kg)

Group IV. *Monochoria vaginalis* (500 mg/kg), orally for 14 days + DOX (20 mg/kg) single intraperitoneal injection after 14th day

Group V. (Extract control). *Monochoria vaginalis* (500 mg/kg), orally for 14 days. single intraperitoneal injection after 14th day

Isolation of working heart preparation: The animals were anesthetized with chloroform after 72 h of DOX administration, and then heart
was punctured with a sterile syringe and blood was stored with EDTA, which is an anticoagulant agent and was excised out. Cardiac muscle from the lower third of the ventricle was collected and stored in liquid nitrogen for antioxidant studies.

INVITRO ANTIOXIDANT ACTIVITY

DPPH radical scavenging activity: Free radical scavenging ability of the extracts was tested by DPPH radical scavenging assay as described by Blois,*et al. al*., 1958 and Desmarchelier *et al* 1997 [7-8]. The hydrogen atom donating ability of the plant extract was determined by the decolorization of methanol solution of 2,2-diphenyl- 1-picrylhydrazyl (DPPH). DPPH produces violet/ purple color in methanol solution and fades to shades of yellow color in the presence of antioxidants. A solution of 0.1 mM DPPH in methanol was prepared, and 2.4 mL of this solution was mixed with 1.6 mL of extract in methanol at different concentrations (15–240 µg/mL). The reaction mixture was vortexed thoroughly and left in the dark at RT for 30 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. BHT was used as reference. Ascorbic acid was used as reference substance.

Reducing Power: The reducing power of sample was determined to the reported method [9]. The five concentration of leaf extracts (15, 30, 60, 120, 240 µg/mL) in 1 ml of distilled water were mixed with phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 min. A portion (2.5 ml) of TCA (10%) was added to the mixture, which was then centrifuged for 10 min at 1000 X g (MSE Mistral 2000, U.K). The upper layer of solution (2.5 ml) was mixed with distilled water (2.5ml) and Ferric chloride (0.5 ml, 0.1%), and absorbance as measured at 700nm in spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

Superoxide anion Scavenging Activity

The superoxide anion scavenging activity was measured as described by Srinivasan *et al* [10]. The superoxide anion radicals were generated in 3.0 ml of Tris – HCL buffer (16 mM, pH 8.0), containing 0.5 mL of NBT (0.3mM), 0.5 ml NADH (0.936mM) solution, 1.0 mL extract of different concentration (15 – 240 µg/ml), and 0.5 mL Tris – HCl buffer (16mM, PH 8.0). The reaction was started by adding 0.5 mL PMS solution (0.12mM) to the mixture, incubated at 25ºC for 5 min and the absorbance was measured at 560 nm against a blank sample; ascorbic acid was used as standard.

The percentage inhibition was calculated by using the following equation Superoxide radical scavenging activity= {(A0 –A1)/A0)*100}

Where, A0 is the absorbance of the control reaction, and A1 is the absorbance in presence of all of the extract samples and reference.

Scavenging of Hydrogen Peroxide: Hydrogen peroxide scavenging potential of Monochoria Vaginalis was determined using the method described by Jayaprakasha *et al*. [11]. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS, pH 7.4). Different concentrations of the extract (15 to 240 µg/ml) in ethanol (1 ml) were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min the absorbance was measured at 230 nm against a blank solution that contained hydrogen peroxide solution without the extract. The percentage of H_2O_2 scavenging of the plant extract was calculated as follows:

% Scavenged hydrogen peroxide = $[(A_0 – A_1)/ A_0]$ X 100

Where A0 – Absorbance of Control, A_1- Absorbance of samples and standards.

Nitric oxide Radical Scavenging Activity: Nitric oxide scavenging activity was measured by using a spectrophotometer. Sodium nitroprusside (5 mM) in phosphate buffered saline was mixed with different concentrations of *Monochoria vaginalis* and ascorbic acid as std (15 – 240 µg/mL) dissolved in methanol and incubated at 25°C for 30 min. A control without test compound but with equivalent amount of methanol was taken. After 30 min 1.5 ml of the incubation solution were removed and diluted with 1.5 ml of Griess reagent (1% sulphanilamide and 0.1% napthyl ethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with naphthyl ethylene diamine was measured at 546 nm [12].

BIOCHEMICAL ANALYSIS

Heart homogenate was used for the estimation of Ca-ATPase, phospholipase, lipid peroxide, phospholipid, glycogen and cytosolic xanthine oxidase was estimated by the method of Kaul, [13-14].

Statistical analysis: The obtained results were analyzed for statistical significance using one way ANOVA followed by Dunnet test using graphpad prism software statistical software for comparison with control group and DOX treated group. $P<0.05$ was considered as significant.

RESULTS AND DISCUSSION

GC MS/MS analysis: The ethanolic extract of *monochoria vaginalis* is a complex mixture of many constituents and 16 compounds were identified in this plant by GC–MS/MS (Fig.1, Table 1). 1-Dodecanol, 3,7,11-trimethyl- , 2-Buten-

1-imine, N-cyclohexyl-4-(2-fluoro-2-methylcyclohexyl)-, N-oxide, α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl, Phenol, 2,4-bis(1,1-dimethylethyl)-, 18-Nonadecenoic acid, 3-Buten-2-one, 3-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)-, Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1,2-Benzenedicarboxylic acid, butyl octyl ester, Hexadecanoic acid, methyl ester, 1(2H)-Naphthalenone, 3,4,4aα,5,6,7,8,8a-octahydro-5,5,8aβ-trimethyl-, (2,4-dinitrophenyl)hydrazone, 9,12-Octadecadienoic acid (Z,Z)-, methyl ester, 10-Octadecenoic acid, methyl ester, Didodecyl phthalate, Squalane and 1-Monolinoleoylglycerol trimethylsilyl ether were identified in the ethanol extract of *monochoria vaginalis* by relating to the corresponding peak area through coupled GC–MS . Most of the phytochemical compounds possess medicinal properties (eg.Antioxidant, Lipoxygenase-inhibitor antimicrobial, Hypocholesterolemic anti-tumor, and antiinflammatory properties, as identified by Dr.Duke's Phytochemical and Ethnobotanical Databases).

Acute toxicity: It was observed that the administration of single oral dose 2000 mg/kg/body weight of ethanol extract of *Monochoria Vaginalis* to a rat, didn't induce drug related toxicity and mortality in the animals and it was safe up to the dose of 2000mg/kg/body weight.

Invitro antioxidant activity: Preliminary Phytochemical analysis gave positive results for steroids, triterpenoids and fluro and amide compounds (Table 1& Figure 1). Further extracts samples were screened for their possible antiradical activity by four complimentary systems namely DPPH, Nitric acid, Superoxide and Hydrogen peroxide systems. Free radical scavenging capacities of the extracts shown in (Figure 2). For the measurement of reducing ability the Fe^{3+}-Fe^{2+} transformation was investigated in the presence of both samples (Figure. 3) depicts the reductive effects of MV compared with ascorbic acid.

For the measurement of Super Oxide anion radical scavenging activity (Figure 4), Hydrogen peroxide scavenging activity (Figure 5), Nitric oxide scavenging activity (Figure 6) was investigated in the presence of both samples showed the reductive effects of MV extract compared with ascorbic acid.

Reactive oxygen species (ROS) such as superoxide radicals, hydroxyl radicals, iron- oxygen complexes, hydrogen peroxide and lipid peroxides are generated by several oxidative reactions [15]. Although ROS can help the immune system clean out extrusive microorganisms, excessive ROS can also react with biological molecules such as DNA, proteins and phospholipids, and eventually cause oxidative damage in tissue and free radical- related diseases such as inflammation, heart disease, diabetes, gout , cancer, etc [16]. For aerobic organisms, the major system of defense against oxidative damage is the use of "antioxidant" enzymes to convert excessive ROS into non-toxic compounds [17&18].

Many plants exhibit efficient antioxidant properties owing to their phenolic constituents. Most of tannins and flavonoids are phenolic compounds and may be responsible for antioxidant properties of many plants [19]. In MV extract also exhibit phenolic compound, therefore it has better antioxidant potentials as compared with previous studies.

The reducing power of bioactive compounds had been reported to be associated with their antioxidant effect. Our data on the reducing power of extracts suggested that it was likely to contribute significantly towards the observed antioxidant effect. (Figure 3)

A significant correlation was obtained between the total phenols in the extracts and the inhibition of lipid peroxidation by the extracts. We believe that the antioxidant activity shown by the extracts probably due to the presence of phenolic compounds. Our results are in agreement with those that reported the ability of phenolic compounds to scavenge free radicals and active oxygen species [20].

Hydrogen peroxide itself is not very reactive, but it can be toxic some time to cells because of it may give rise to a hydroxyl radical [21]. Thus removing hydrogen peroxide is very important for protection of biological systems. Scavenging of hydrogen peroxide by *Mo*nochoria vaginalis may be attributed to their phenolics, which could donate electrons to hydrogen peroxide, thus neutralizing it to water.

Super oxide radical is known to be very harmful to cellular components as a precursor of more reactive oxidative species, such as single oxygen and hydroxyl radicals [22]. Furthermore, superoxide radical is considered to play an important role in the peroxidation of lipids [23]. Therefore studying the scavenging effects of plant *Monochoria vaginalis* on superoxide radicals is one of the most important ways of clarifying the mechanism of anti-oxidant activity.

BIOCHEMICAL PARAMETERS

Effect of *Monochoria Vaginalis* on Myocardial Infraction: Administration of doxorubicin to induce ischemia heart there was a significant reduction in levels of Ca-ATPase (Figure 7), glycogen and phospholipids (Figure 8) in Group II as compared to Group I. However there was a significant increase in Phospholipase and xanthine oxidase (Figure 7) along with increase in lipid peroxide (Figure 8) in DOX induced rats (Group II) Whereas treatment with monochoria vaginalis extract significantly decreased the Phospholipase and xanthine oxidase (Group IV) and significantly decreased the lipid peroxide activity (Group III & IV) as compared to the levels of induced group (Group II).

Animals treated with doxorubicin (Group II) produced significantly decrease in Ca2+- ATPase when compared with Group I are shown in Figure 7. In drug treated groups III and IV. After the treatment with MV extract the levels are significantly increased when compared with group II.

The current investigation revealed that doxorubicin showed significantly decreased in activity of membrane bound enzymes. Membrane bound ATPases of cardiac cells play a significant role in contraction and relaxation cycles of cardiac muscle by maintaining normal ion levels within the myocytes. Changes in the properties of this ion pumps affect cardiac function. It was reported that inhibition of Ca2+ ATPase induced pertubation of cell calcium homeostatis and this effect may associated with increase Ca2+ permeability [24].

In the heart cytosolic calcium accumulation may be a relevant mechanism leading to cell death and has been proposed to play an important role in pathogenesis of lethal myocardial cellular injury. The restoration of membrane bound enzyme Ca2+ ATPase in rats is indicative of membrane stabilizing protective effect of MV extract.

The increase in xanthine oxidase and lipid peroxide in damaged rat heart was protected significantly by the treatment with monochoria vagilnalis extract. It has been reported that xanthine dehydrogenase is converted into xanthine oxidase under myocardial infarction that at the moment of reoxygenation (respiratory burst) produces O2- and uric acid. The O2- and H2O2 are the main source of OH-, which play an important role in cardiac necrosis [25].

CONCLUSION

The results clearly indicate that the *Monochoria vaginalis* extract has highest antioxidant activity against various radicals in *vitro*. It can be used as easily accessible source of natural antioxidants in food as vegetables. However, the compounds responsible for the antioxidant activity have not been identified. Further study is currently under way aimed towards the isolation and characterization of the compound which is responsible for antioxidant activity. These facts can be further exploited, for instance, in obtaining a bioactive graded fraction, which will have improved antioxidant activity as compared to crude extract *monochoria vaginalis*.

Our study shows that ethanolic extract of *Monochoria vaginalis* possess marked cardioprotective activity and play a promising role in treating the myocardial infarction caused by DOX. The cardioprotective activity is demonstrated by changes in the heart tissue homogenate xanthine oxidase phospho lipase, phospho lipid, glycogen and lipid peroxidase to the normal level. All the above findings indicate that the *monochoria vaginalis* extract have good cardioprotective activity against DOX induced cardiotoxicity. Further studies will be carried out to find the herbal compound that responsible for protective action against DOX induced cardiotoxicity.

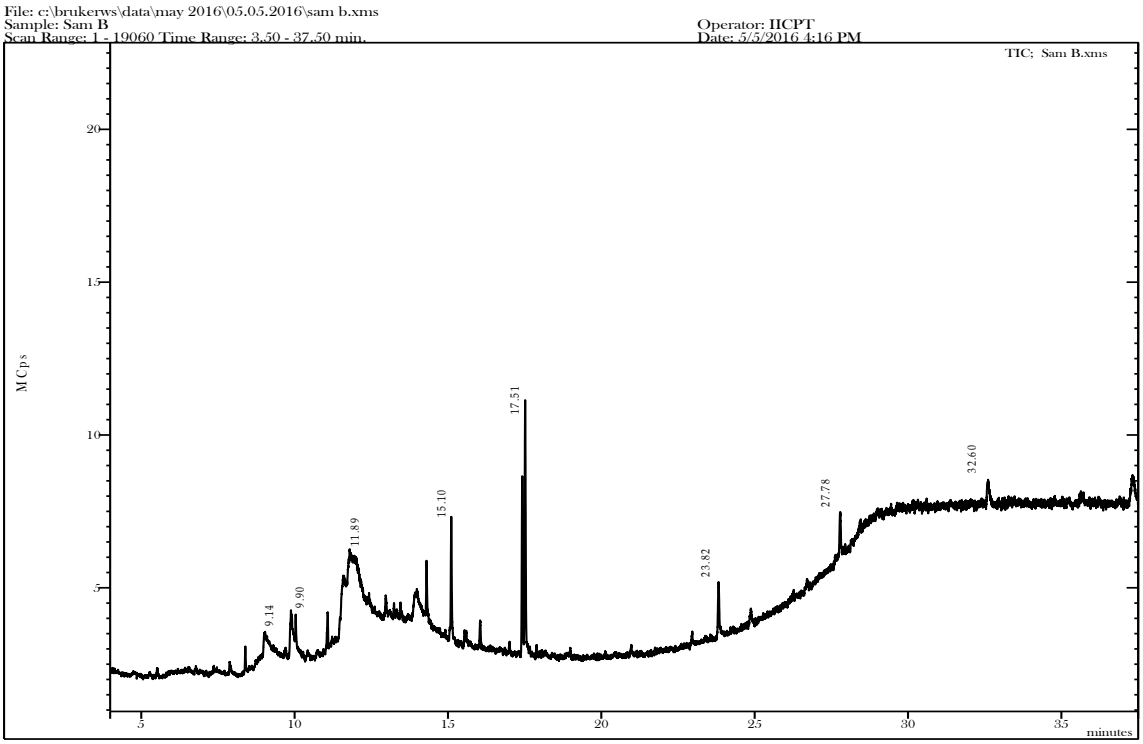

Fig. 1: The chromatogram showing high peaks like 18-Nonadecenoic acid(5.20), Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-(5.53), 1,2-Benzenedicarboxylic acid, butyl octyl ester (9.60), Hexadecanoic acid, methyl ester (11.87), 9,12-Octadecadienoic acid (Z,Z)-, methyl ester (12.52), 10-Octadecenoic acid, methyl ester (20.32) peaks detected by GC MS/MS.

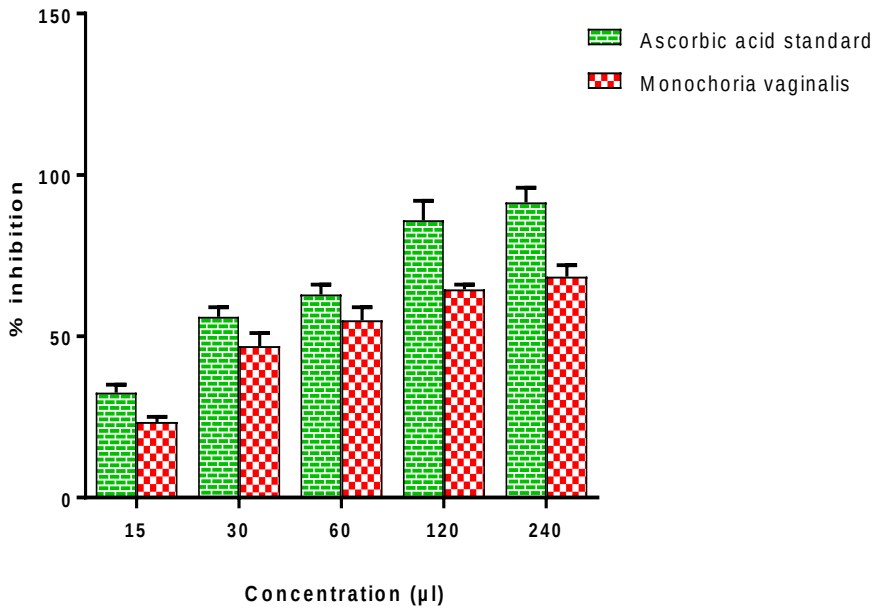

Fig 2 Effect of Monochoria Vaginalis and ascorbic acid on DPPH radical scavenging activity. The values are expressed as mean of three experiments.

Table 1: Phytocomponents identified in *Monochoria Vagilnalis* (GC–MS/MS study)

RT	Name of the compound	Molecular Formulae	MW	Peak Area %	Compound Nature	**Activity
8.39	1-Dodecanol, 3,7,11-trimethyl-	C15H32O	228	1.40	Alcoholic compound	Antimicrobial
9.14	2-Buten-1-imine, N-cyclohexyl-4-(2-fluoro-2-methylcyclohexyl)-, N-oxide	C17H28FNO	281	2.10	Fluro compound	Antimicrobial Narcotic Antitumor Myo-neuro-stimulant
9.90	α-D-Glucopyranoside, O-α-D-glucopyranosyl-(1.fwdarw.3)-β-D-fructofuranosyl	C18H32O16	504	1.88	Glucose moiety	Anticancer Antidote for Organo phosphorus
10.04	Phenol, 2,4-bis(1,1-dimethylethyl)-	C14H22O	206	2.65	Phenolic compound	Antimicrobial Anti-inflammatory Antioxidant Analgesic
11.07	18-Nonadecenoic acid	C19H36O2	296	5.20	Unsaturated fatty acid	No activity reported
11.89	3-Buten-2-one, 3-methyl-4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]heptan-1-yl)-	C14H22O2	222	4.53	Ketone compound	No activity reported
12.97	Acetamide, N-methyl-N-[4-(3-hydroxypyrrolidinyl)-2-butynyl]-	C11H18N2O2	210	5.32	Amide compound	Antimicrobial
13.98	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	296	2.88	Terpene alcohol	Antimicrobial Anti-inflammatory Oligosaccharide Provider
14.29	1,2-Benzenedicarboxylic acid, butyl octyl ester	C20H30O4	334	9.60	Plasticizer compound	Antimicrobial Anti-fouling
15.10	Hexadecanoic acid, methyl ester	C17H34O2	270	11.87	Palmitic acid ester	Antioxidant Hypocholesterolemic Nematicide Pesticide Lubricant Antiandrogenic Flavor Hemolytic 9.5-Alpha reductase inhibitor
16.04	1(2H)-Naphthalenone, 3,4,4aα,5,6,7,8,8a-octahydro-5,5,8aβ-trimethyl-, (2,4-dinitrophenyl)hydrazone	C19H26N4O4	374	3.37	Poly aromatic compound	Antidote for heavy metals

17.41	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	C19H34O2	294	12.52	Linoleic acid ester	Anti-inflammatory, Hypocholesterolemic Cancer preventive, Hepatoprotective, Nematicide Insectifuge, Antihistaminic Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic, Antiarthritic, Anticoronary, Insectifuge
17.51	10-Octadecenoic acid, methyl ester	C19H36O2	296	20.32	Unsaturated fatty acid	No activity reported
23.82	Didodecyl phthalate	C32H54O4	502	5.40	Plasticizer compound	Antimicrobial Anti-fouling
27.78	Squalane	C30H62	422	7.11	Triterpene	Antibacterial, Antioxidant, Antitumor, Cancer preventive, Immunostimulant, Chemo preventive, Lipoxygenase-inhibitor, Pesticide
32.60	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498	3.86	Ether compound	No activity reported

**Source: Dr.Duke's Phytochemical and Ethno botanical Databases

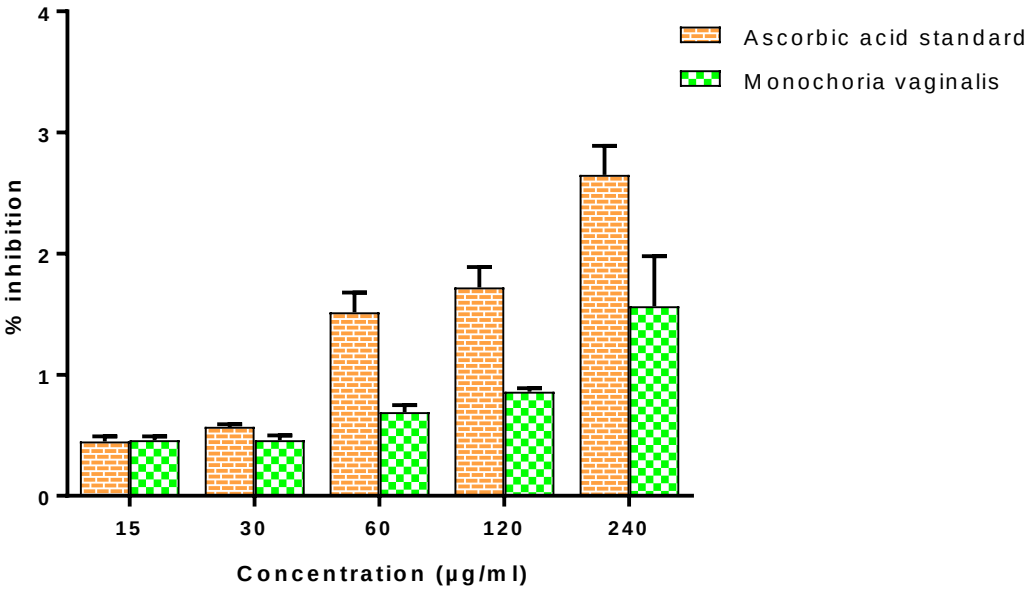

Figure 3. Effect of Monochoria Vaginalis and ascorbic acid on reducing power.

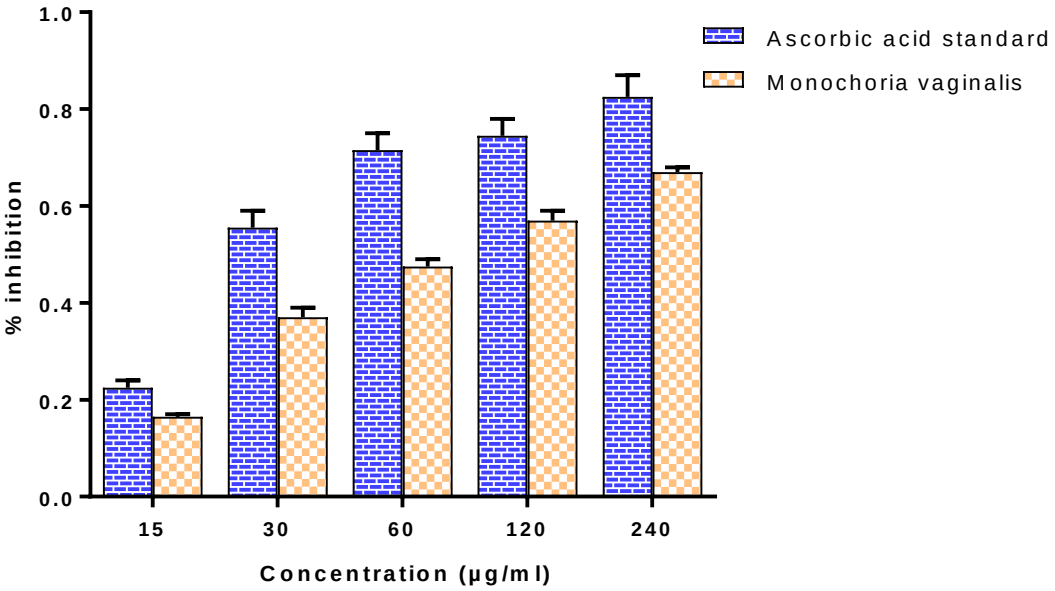

Fig 4. Effect of *Monochoria Vaginalis* and ascorbic acid on Super Oxide anion radical scavenging activity.

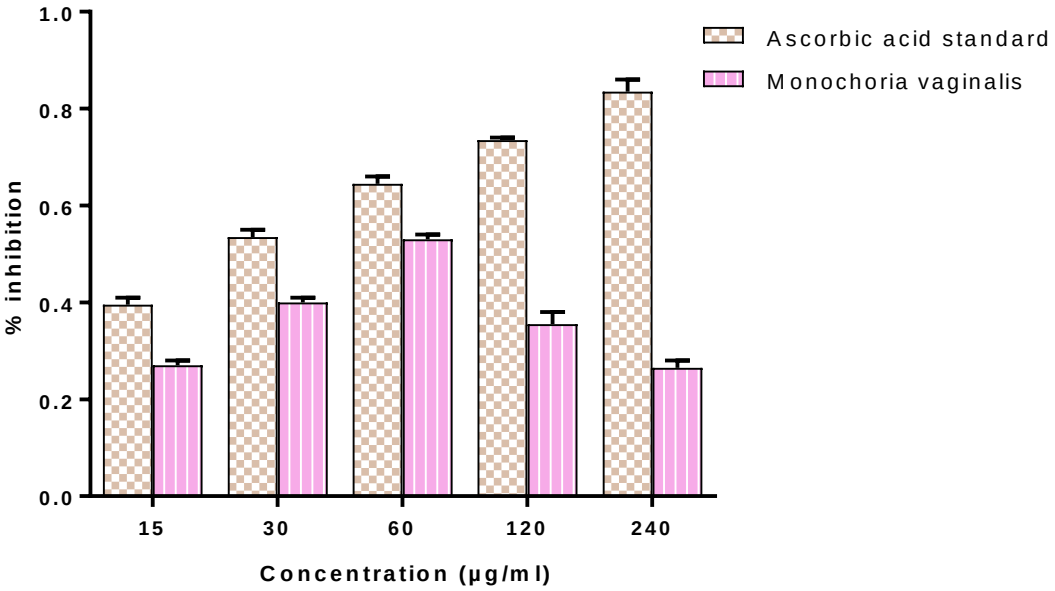

Fig 5 Figure 13: Effect of *Monochoria Vaginalis* and ascorbic acid on Hydrogen peroxide scavenging activity.

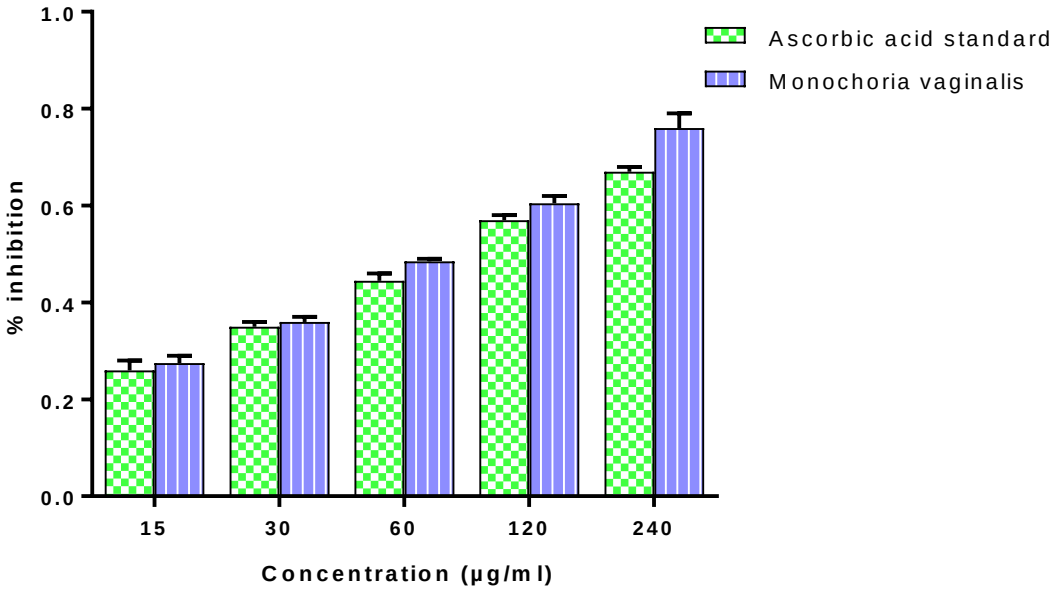

Figure 6: Effect of *Monochoria Vaginalis* and ascorbic acid on Nitric oxide scavenging activity.

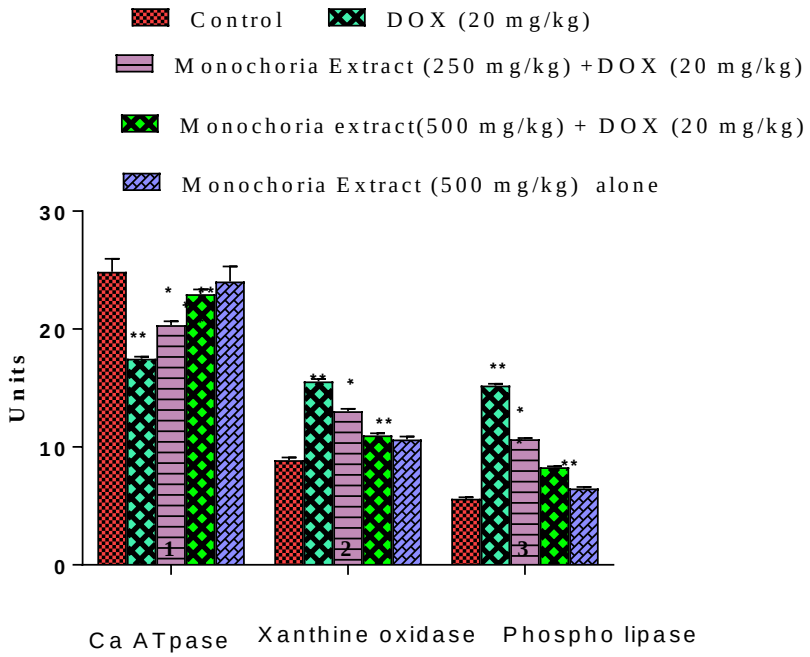

Figure 7: Effect of ethanolic extract *Monochoria vaginalis* on Ca-ATPase (µ mole Pi/hr/mg protein) **Xanthine oxidase** (µmole uric acid/ min/ mg protein) , Phospholipase (µmole FFA/hr/mg protein) in DOX intoxicated rats. Values are mean ± S.D (n=6) *P<0.01, **P<0.05 respectively.

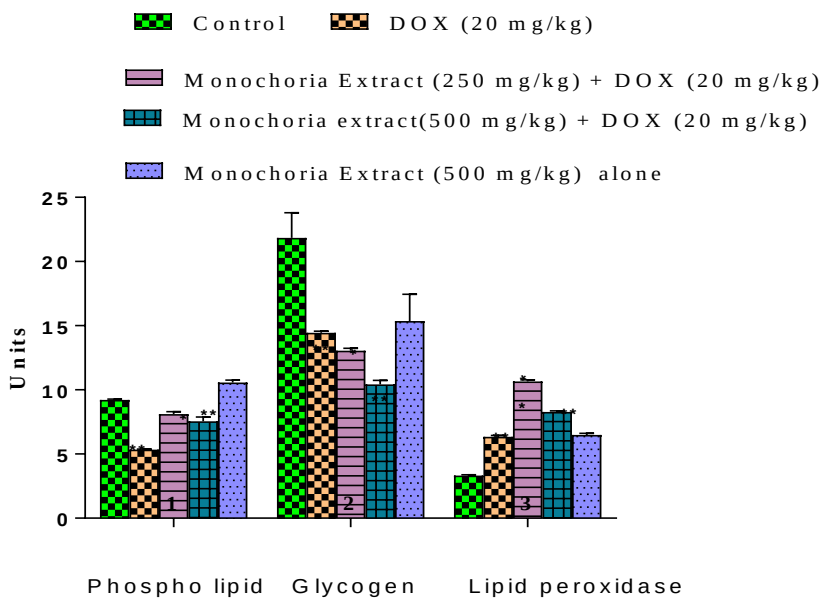

Figure 8: Effect of ethanolic extract *Monochoria vaginalis* on Phospho lipid , Glycogen and Lipid peroxidase (mg/gm) in DOX intoxicated rats. Values are mean ± S.D (n=6) *P<0.01, **P<0.05 respectively.

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