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Evaluation of Bioactive Compounds from the Fresh Water Weed *Cabomba caroliniana* A. Gray

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ABSTRACT

The exotic freshwater weed Cabomba caroliniana A. Gray is found to have a fast spread in wetlands of Kerala (India), posing a threat to the inland water bodies. The present work was undertaken to characterize the bioactive molecules from Cabomba, in order to explore the potent possibilities of utilizing the plant as a medicinal resource. Dried powder of Cabomba caroliniana was extracted in solvents such as 80% ethanol, methanol, acetone, distilled water, chloroform and petroleum ether and the crude extract tested for in-vitro anti-inflammatory, antioxidant, MMP inhibitory and antimicrobial properties. GC-MS analysis of acetone extract of the plant was done using Varian CP3800 GC system. The study indicated that various solvent extracts of Cabomba exhibited the degree of MMP inhibition in the order, methanol>ethanol>distilled water>acetone>chloroform>petroleum ether. Cabomba extract was found to inhibit the heat induced heamolysis in RBCs to varying degrees; acetone and methanol extracts were found to significantly protect the erythrocyte membrane against lysis induced by heat. The free radical scavenging potential of extract was found to be maximum in acetone (66.22%), followed by methanol (65.56%), ethanol (63.15%) and distilled water (60.92%). The antimicrobial activity of acetone extract of the plant at 800 µg ml^{-1} was found to be most pronounced against all test microorganisms under study. Acetone and methanol were found to be the most effective solvents; 600 to 800 µg ml^{-1} could be considered as the optimum concentration range. The GC-MS analysis of acetone extract of Cabomba showed the presence of thirteen compounds, of which five were prominent. The aquatic weed Cabomba caroliniana is endowed with many potential phytochemicals, which could be effectively utilized in the production of newer plant based drugs.

Keywords: *Cabomba caroliniana*; Aquatic weeds; Bioactive compounds

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INTRODUCTION

The search for new molecules, nowadays has taken a slightly different route where the science of ethno-botany and ethno pharmacognasy are being used as guide to lead the chemists towards different sources and classes of compounds. It is believed that there are many producers of natural compounds unexplored in the aquatic environment that could become potential sources for the reduction or control of bacterial diseases [1]. The antibacterial activity observed in some water plants has motivated researchers to work with other species as well [2]. A variety of aquatic plants are used in curative therapy by traditional communities. A good number of these ethno-botanic materials have been reported to yield compounds which could be of use as modern drugs and pharmaceuticals [3]. One advantage in studying aquatic plants is their high growth rate, which facilitates large scale production of extracts for purpose of purification of active compounds [4,5].

Cabomba caroliniana, commonly known as Green *Cabomba*, is an aquatic perennial herbaceous plant native to North America, a long-lived, mostly underwater plant with stems that are often covered with a thin layer of jelly-like slime. It's oppositely arranged underwater leaves (3-7 cm in size) are finely divided and are fan-shaped in appearance. Its inconspicuous floating leaves are small (up to 20 mm long), narrowly oval or elongate in shape, having entire margins. Its small white flowers (6-20 mm across) have a yellowish centre and are borne just above the water surface. *Cabomba caroliniana* is regarded as one of the worst aquatic weeds in many places and is of significance, largely because of its invasiveness, potential for spread, and environmental impacts (Figure 1a and 1b) *Cabomba caroliniana* is found to have fast spreading in wetlands of Kerala. Kerala state of peninsular India, in addition to having a number of rivers traversing the state from east to west supports numerous man-made canals crisscrossing the low-land belt, connecting various rivers. The irrigation canals form a network connecting rivers and coastal lagoons throughout the length of the state; all these freshwater bodies are found to support a number of aquatic weeds. *Cabomba caroliniana* is found to have fast spreading, especially in the Vembanad region, growing in all types' water bodies-canals, rivers, lakes and paddy fields. Studies have reported that the growth of the plant along Pampa course at Aranmula is a threat to river system as a whole. The plant with the external dense strands which obstructs the free flow of water is found to increase silting of river making it shallow and dry. *Cabomba* can clog waterways to the extent that it reduces water storage capacity, interferes with pumping equipment and disrupts drinking water supplies. Taking into consideration the above mentioned facts and also potential therapeutic value of aquatic macrophytes, the present work was undertaken to characterize the bioactive molecules from freshwater macrophyte *Cabomba caroliniana* A. Gray so as to enable a two way process-eradication of the weed and also its utilization for production of novel and useful compounds.

MATERIALS AND METHODS

Location and Collection of Plant

The whole plant of *Cabomba caroliniana* used for the present research work was collected during the months of April-May from Vembanad Lake in Kavalam, Kerala, India (Figure 2). The plant was identified using standard flora [6-8] and deposited in the herbarium, Centre for Sustainable Aquatic Resources and Algal Biotechnology (C-SARAB), School of Biosciences, Mar Athanasios College for Advanced Studies, Tiruvalla, Kerala, India. After collection, the fresh water plant material was transported to the laboratory. In the laboratory the plant was first

rinsed thoroughly in tap water to remove the attached debris and epiphytes followed by distilled water. The plant material was ground to a fine powder (<300 µ) and stored in airtight containers until the time of extraction.

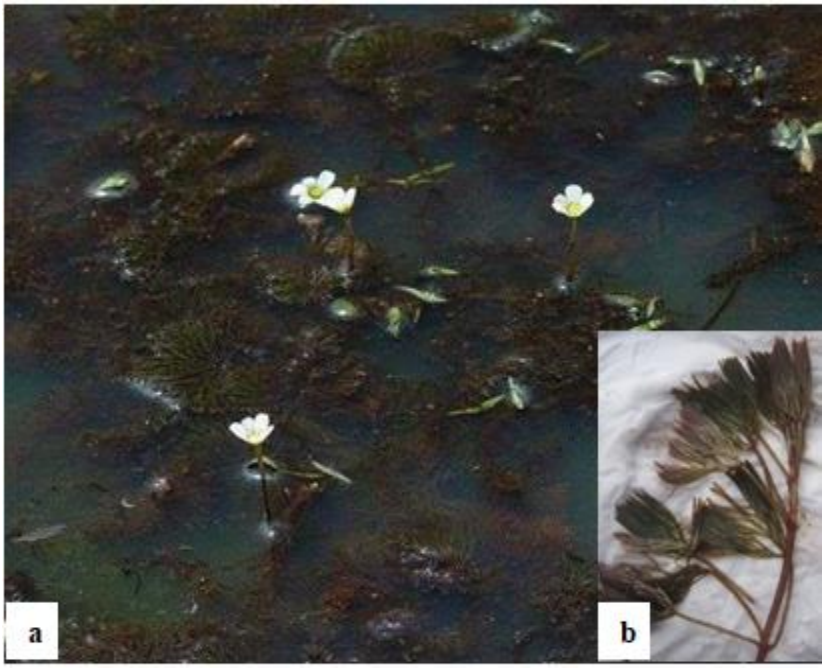

Figure 1. (a) *Cabomba caroliniana* with flowers; (b). Thallus of *Cabomba caroliniana*

Figure 2. Map showing collection site

Preparation of Plant Extracts

For preparing the plant extract, 3 g powdered samples in 100 ml each of solvents such as 80% ethanol, methanol, acetone, distilled water, chloroform and petroleum ether were taken and kept on a shaker (120 rpm) for 24 hrs at room temperature. Each mixture was then centrifuged at 3000 xg for 15 minutes (REMI R-8C Lab Centrifuge) and the supernatant collected. The different extracts were concentrated to dryness using a rotary evaporator (Superfit^{TM}) at 60°C and weights of the residues noted. The dried extracts were re-dissolved in above mentioned respective solvents (anti-inflammatory assays) to get concentrations of 100 µg ml^{-1}, 200 µg ml^{-1}, 400 µg ml^{-1},600 µg ml^{-1} and 800 µg ml^{-1}, in methanol for antioxidant assay. For the MMP inhibitory and antimicrobial studies, the dried extracts were dissolved in DMSO to get the above concentrations.

Assessment of *in vitro* Anti-inflammatory Activity

Membrane stabilization test

Preparation of drug: Standard drug (Indomethacin, 2.5 mg/ml) and extracts in respective solvents (100, 200, 400, 600 and 800 µml^{-1} concentrations) was prepared in isosaline (0.85% NaCl) to final the concentration.

Preparation of Suspension (40% v/v) of human red blood cell: The blood sample was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment and transferred to heparinized centrifuge tube. Blood samples were centrifuged at 3000 rpm at room temperature for 15 min. The supernatant (plasma and leucocytes) were carefully removed while the packed red blood cell was washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation were repeated five times until the supernatants were clear. Then, Human erythrocytes suspension (40% v/v) was prepared as reported by Oyedapo et al. [9].

Hypotonic Solution-induced Haemolysis Or membrane Stabilizing Activity

The HRBC membrane stabilizing activity assay was carried out as reported [9,10] using 10% (v/v) Human erythrocyte suspension while Indomethacin was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline.

To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were used.

1. Test solution (4.5 ml) consists of 2 ml of hypotonic saline (0.25%w/v), 1 ml of phosphate buffer (pH 7.4), 1 ml of test extract (100 µg ml^{-1}, 200 µg ml^{-1},400 µg ml^{-1} 600 µg ml^{-1},800 µg ml^{-1}) and 0.5 ml of 10% w/v human red blood cells in isotonic saline.
2. Test control (4.5 ml) consists of 2 ml of hypotonic saline (0.25%w/v) 1 ml of phosphate buffer (7.4 pH) and 1 ml of isotonic saline and 0.5 ml of 10%w/v human red blood cells in isotonic saline.
3. Standard solution (4.5 ml) consists of 2 ml of hypotonic saline (0.25%w/v) 1 ml of phosphate buffer (7.4 pH) and 1 ml of Indomethacin (2.5 mg/ml) and 0.5 ml 10%w/v human red blood cells suspension, in isotonic saline.

Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate

and the average value was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation.

$$\% \text{ Inhibition of haemolysis} = \left(A1 - A2 / A1\right) \times 100$$

Where:

A1=Absorption of hypotonic buffered saline solution alone

A2=Absorption of test sample in hypotonic solution

Assessment of *in vitro* Anti-oxidant Activity

DPPH assay (1,1-diphenyl-2-picrylhydrazyl): DPPH in methanol (0.1 mM) was prepared and 1.0 ml of this solution was added to 3.0 ml of extract solution in methanol at different concentrations. Thirty minutes later, the absorbance was measured at 520 nm. A blank was prepared without adding extract. Ascorbic acid at concentration 50 µg ml^{-1} was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged}\left(\%\right) = \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

where A control is the absorbance of the control reaction and A test is the absorbance in the presence of the sample of the extracts.

Assessment of Anti-Arthritic activity

Gelatin digestion assay

Agarose solution (1%) was prepared in collagenase buffer (50 mM tris-Hcl, 10 mM Cacl, 0.15 M NaCl, 7.8 pH) with 1.5% porcine gelatin (Sigma Aldrich) and allowed to solidify in wells of 12 well plate (2 ml/ well) for 1 hr at room temperature. Different concentrations of *Cabomba* extract (10 µl) were incubated with 5 µl of Synovial fluid (of osteoarthritic patients, collected from Pushpagiri Medical Mission Hospital, Tiruvalla, Kerala) in 85 µl of collagenase buffer for 24 hrs. The reaction products (10 µl) were loaded onto paper disks placed on gelatin-agarose gel and incubated for 18 h at 37°C, the degree of gelatin digestion in agarose gel was visualized by Coomassie Brilliant blue staining after removal of paper discs. Following destaining, the area of light translucent zone of blue background was examined to estimate gelatinase activity. Three replicates were kept for each case and averages of the results were taken. 10 µl of pure arthritic fluid was taken as negative control and 10 µl of Doxycyclin (Microdox, Micro Labs Ltd., India) as positive control. The colour intensity was estimated using Image J Software, 1.45 K version (www.softonic.com) and the percentage inhibition of MMPs was calculated using following formula,

$$\% \text{ Inhibition of MMPs} = *100 - \frac{\text{A control} - \text{A test}}{\text{A control}} \times 100$$

A control=Color intensity of positive control (Doxycycline)

A test=color intensity of test samples

(*% inhibition by Doxycycline is taken as 100).

Gelatin Zymography

Different concentration of *Cabomba* extracts incubated with synovial fluid (as in gelatin digestion assay) was suspended in a sample buffer (125 mM tris-HCl, pH-6.8, 3% SDS, 40% glycerol, 0.02% Bromophenol blue) without boiling and electrophoresed under non-reducing conditions on 10% polyacrylamide gel containing 1.5 mg/ml gelatin. After electrophoresis, the gel was washed twice with 50 mM Tris-HCL (pH-7.5) containing 2.5% triton X-100 and incubated overnight at 37°C in a developing buffer (10 mM CaCl, 50 mM Tris-HCL, and 150 mM NaCl). The gels were stained with 0.25% Coomassie Brilliant Blue R-250 in 30% methanol and 10% acetic acid and distained in the same solution without dye. Gelatinolytic bands were observed as clear zones against the blue background and the inhibitory effect of Cabomba extract was assessed from the intensity of the bands (Figure 3). Gelatin digestion assay and gelatin zymography were carried out based on the method given by Kim et al. [11].

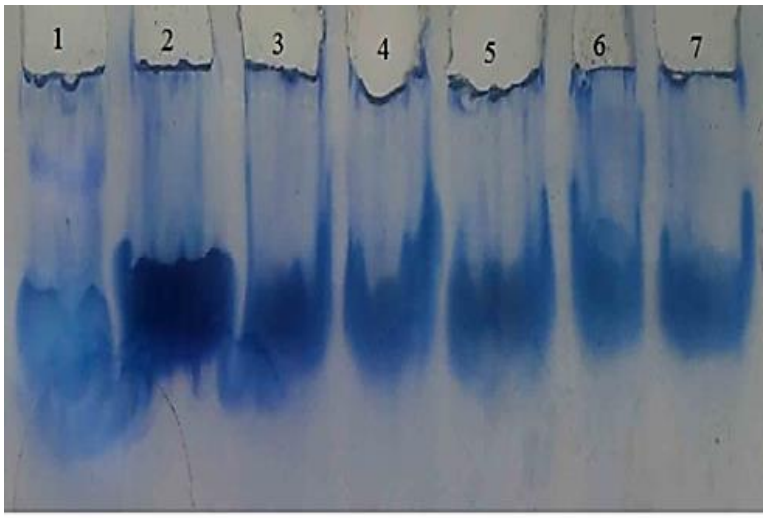

Figure 3. Gelatin Zymography of *Cabomba* extract

SDS-PAGE

SDS-PAGE gel electrophoresis was performed on resolving and stacking gel at 10% and 4% concentrations of acrylamide: bis-acrylamide respectively. The MW (Da) of protein was determined by using standard molecular weight markers (Aristogene Biosciences). 10 µl of sample buffer (125 mM tris Hcl (pH 6.8), 40 % (v/v) glycerol, 3% (w/v) SDS and 0.1% (w/v) Bromophenol Blue) added to 10 µl of synovial fluid and molecular weight marker separately. The 20 µl of each mixture was injected to the stacking gel. The electrophoresis unit (Indus Biosolution) was filled with the 1X running buffer and the electrophoresis was carried out at 100 V. After electrophoresis gels were placed in the staining solution for 4-6 hr at 37°C with agitation, followed by its destaining in the destaining solution with agitation. The destained gels were immediately photographed and protein bands were analyzed, comparison of bands obtained for synovial fluid was compared with that of marker protein to determine the MMPs present.

Antimicrobial Assay

The disc diffusion method [12] was adopted for the antimicrobial assays against the pathogen *S. aureus, E. coli, Klebsiella sp. and Pseudomonas sp*. 50 µl each of five different concentrations of the extract (100 µg ml^{-1}, 200 µg $ml^{-1,}$ 400 µg ml^{-1}, 600 µg ml^{-1}, 800 µg ml^{-1}, 800 µg ml^{-1}) obtained from *Cabomba caroliniana* were loaded on to sterile Whatman No. 1 filter paper discs (5 mm dia.) and left to dry for 2-3 hours. The discs were allowed to dry completely before transfer to nutrient agar streaked with 18 hrs old bacterial inoculums using a sterile swab, and left for incubation at 37°C for 24 hrs. Each species was screened in triplicate and averages of the results were considered. 50 µl of pure DMSO was used as a negative control and Gentamycin disc (Himedia) with concentration of 10 mg ml^{-1} was used as positive control [13]. The acetone extract of *Cabomba caroliniana* having good antimicrobial activity on bacteria was further subjected to GC MS analysis for identification of the active components.

GC MS Analysis of Acetone extract of *Cabomba caroliniana*

The GC-MS analysis of Acetone extract of *Cabimas Caroliniana* was carried out using Varian CP 3800 GC system. The detector was Saturn 2200 inert MSD with Ion trap Detector; with column (VF-5MS) 30 m × 0.25 m fused capillary silica tubing. Software adopted to handle mass spectra and chromatograms was National Institute of Standards and Technology MS. 2005 Library. The following temperature protocol was followed for GC/MS detection. Injection port temperature was 250°C and Helium flow rate was 1 ml/min. Oven temperature was programmed from 100°C with an increase of 4°C/min to 270°C and this temperature was held for 20 minutes. The ionization voltage was 70 eV. The samples were injected in split less mode and mass spectral scan range was set at 40-600 (MHZ). The GC/MS characterized the bioactive components of the Acetone extract of *Cabomba Caroliniana*. The fragmentation patterns of the mass spectra were compared (head to tail) with those stored in the NIST Library. The total running time for GC was 36 minutes.

RESULTS AND DISCUSSION

Though synthetic chemicals also have long been used as active agents in reducing the incidence of plant, animal and human diseases, they are costly, have potentially harmful effects on the environment. Therefore, recently, much attention has been directed toward extracts and biologically active compounds isolated from popular plant species. The bioactive potential of aquatic weeds has already been proved [14,15]. These plants that live in nutrient rich medium, with very high bacterial cell density will become overwhelmed by microbial biofilms, if they lack any means of biofilm control [16,17]. For this reason aquatic plants have attracted the interest of researchers and have shown themselves as promising sources of antimicrobial agents [1]. In this context, the present work was undertaken to characterize the bioactive molecules from freshwater macrophyte *Cabomba caroliniana* A. Gray.

Antiarthritic activity/Matrix Metalloproteinases (MMPs) Inhibitory Activity

During the present study, electrophoresis of arthritic fluid using SDS page (Figure 4) confirmed the presence of MMPs in synovial fluid corresponding to that of MMP-9 and MMP-2 of which MMP-2 was predominant. During the present work the inhibiting effect of *Cabomba* extract on MMPs was tested using multi-well digestion assay as well as zymography. During the multi-well digestion as well as the zymography, if the enzyme has considerable activity then there are white areas in the blue back ground indicating the cleavage of gelatin with no protein. On the

other hand if there is inhibition of enzyme activity (brought about by *Cabomba caroliniana* extract), then the bands appears to be blue due to the lack of gelatinolytic activity due to inhibition of MMPs (Figures 3 and 5).

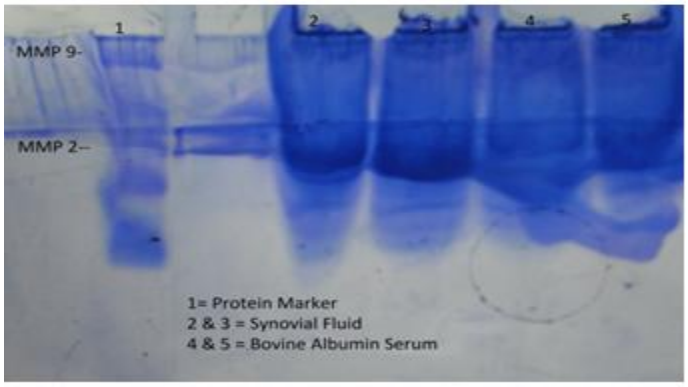

Figure 4. Separation of MMPs with SDS-PAGE

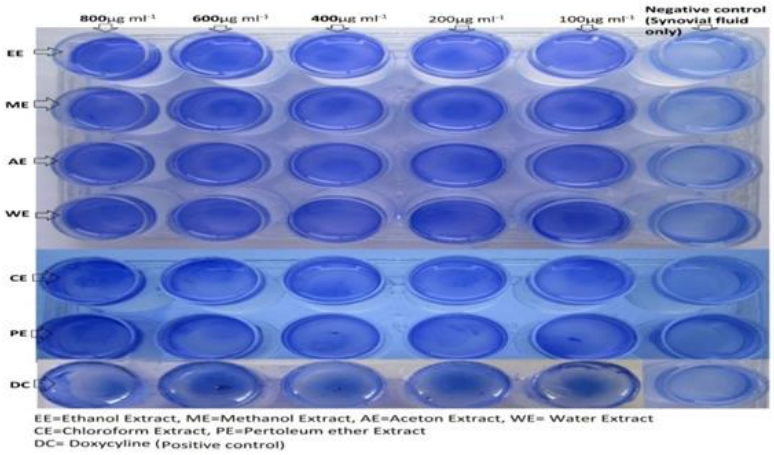

Figure 5. Multi-well gelatin digestion assay with *Cabomba* extract

It clearly indicates that methanol extract of *Cabomba caroliniana* shows high degree of MMP inhibition in both higher and lower concentrations, followed by ethanol, distilled water, acetone, chloroform and petroleum ether (Table 1).

Table 1. Inhibitory effect of *Cabomba caroliniana* extracts toward MMP activity

Samples	Solvents	Degree of Inhibition (%)				
		800 µg ml^{-1}	**600 µg ml^{-1}**	**400 µg ml^{-1}**	**200 µg ml^{-1}**	**100 µg ml^{-1}**
Cabomba caroliniana A Gray	Ethanol	59.33 ± 2	55.67 ± 4	55.95 ± 3	54.5 ± 2	53.85 ± 1
	Methanol	76.3 ± 1	73.09 ± 2	70.03 ± 3	63.99 ± 1	58.7 ± 2
	Acetone	57.28 ± 3	55.06 ± 1	54.66 ± 2	53.42 ± 3	50.04 ± 2
	Distilled water	58.84 ± 1	53.51 ± 2	52.25 ± 1	48.95 ± 4	45.84 ± 4
	Chloroform	51.59 ± 3	47.31 ± 1	46.17 ± 4	40.51 ± 2	34.47 ± 1
	Petroleum ether	48.93 ± 4	46.6 ± 3	44.35 ± 2	40.51 ± 3	35.11 ± 1
Positive control (Doxycyline 500 µg ml^{-1})		100 ± 1				
Negative control (Synovial fluid only)		0				

The degree of inhibition (%), the multi-well gelatin digestion assay, was calculated using software Image J, 1.45k version (www.softonic.com). which is furnished in Figure 6. It can be deciphered that the maximum degree of inhibition is shown by extract in methanol at 800, 600 µg ml^{-1} and 400 µg ml^{-1} concentrations and the average optimum concentration can be taken as 600 µg ml^{-1}. This is comparable to the results of Kim et al., (loc.cit.) who worked on the MMP inhibiting effect of brown algae Ecklonia cava. Ethanol and distilled water extracts exhibit almost similar degree of inhibition in all the five different concentrations. For chloroform extract maximum degree of inhibition is shown at 800 µg ml^{-1} concentration. Comparatively the percentage of inhibition shown by petroleum ether extract was very low, with a maximum inhibition % of 48.93 and minimum of 35.11 at 800 and 100 µg ml^{-1} respectively.

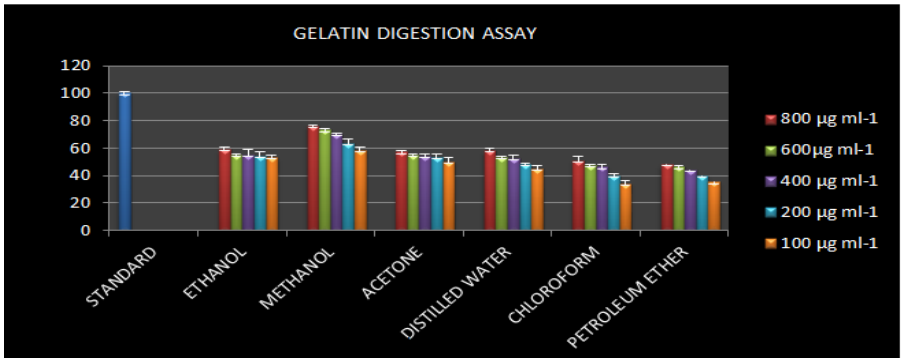

Figure 6. %MMP inhibition of *Cabomba* extract in different solvents

Anti-Inflammatory Test

The HRBC membrane stabilization test has been used as a method to study the *invitro* anti-inflammatory activity because the erythrocyte membrane is analogous to the lysosomal membrane [18], and its stabilization implies that the *Cabomba* extracts may well stabilize lysosomal membranes. This effect may possibly inhibit the release of lysosomal content of neutrophils at the site of inflammation. During the present work, the extracts inhibited the heat induced hemolysis of RBCs to varying degree. The results showed that acetone and methanol extracts at concentrations 600-800 µg ml^{-1} protect significantly the erythrocyte membrane against lyses induced by heat (Figure 7).

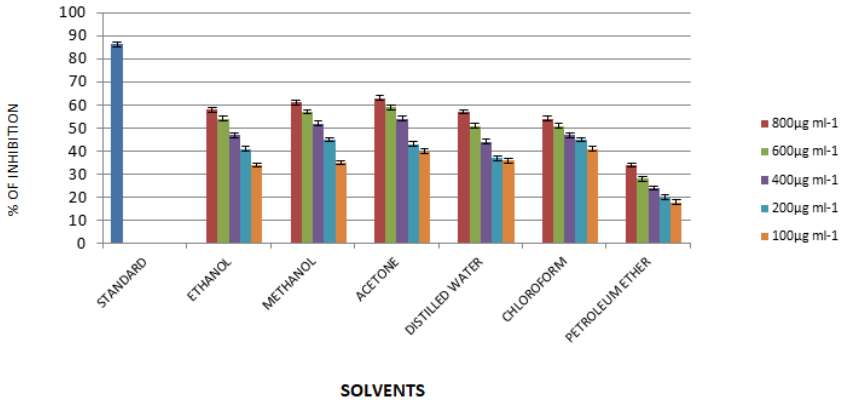

Figure 7. Antiinflammatory effect of *Cabomba* extracts

Antioxidant Activity

Oxidation reactions are necessary part of life, unfortunately they can also be damaging because of the production of Reactive Oxygen Species (ROS). In the past few years, there has been growing interest in the involvement of ROS in several pathological situations [19]. ROS are byproducts of basic metabolic processes, immune reaction against pathogens, air pollution, tobacco smoke, herbicides, and pesticides. In biological systems, phenolic compounds and flavonoids are associated with scavenging ROS [20]. In *invitro* condition DPPH is considered as a stable free radical and it accepts an electron or hydrogen radical to become a stable diamagnetic molecule [21]. The reduction capability of DPPH was determined by the decrease in its absorbance at 520 nm and also by the colour change from purple to yellow, which is induced by anti-oxidants. The degree of discoloration indicates the scavenging potential of the antioxidant compound in the extracts [22]. Antioxidant activity of various extracts of *Cabomba caroliniana* was shown in Figures 7 and 8. Among the different solvents analyzed, acetone extract was found to show the maximum scavenging activity of 66.22% followed by the Methanol, Ethanol and Distilled water extracts with scavenging percentage of 65.56%, 63.15% and 60.92% respectively. Petroleum ether and Chloroform extracts were found to have comparatively lower range of scavenging activity.

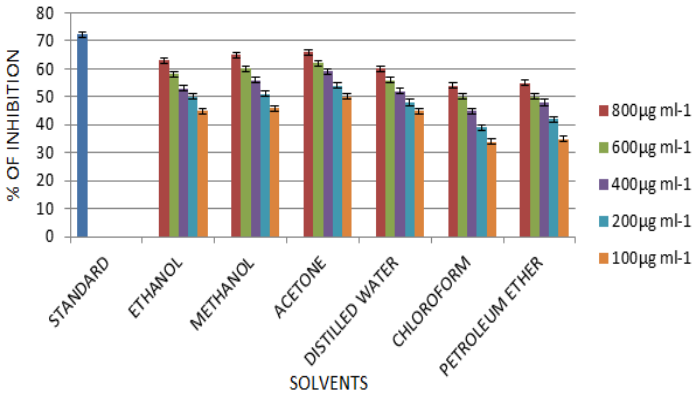

Figure 8. Antioxidant activity of *Cabomba* extracts

Antimicrobial Activity

Although the antimicrobial and other bioactive properties of numerous terrestrial angiosperms, as well as marine macrophytes [23-26] have been studied well, the freshwater angiosperms have received lesser attention [27-39] until recently. During the present work, the acetone extract of the plant proved to have the highest antimicrobial potential. The inhibitory effect was found to be most pronounced in concentrations higher than 500 µg ml^{-1}, and lower concentrations were found to give diminishing activity. The antimicrobial activity exhibited by acetone extract of *Cabomba* in varying concentrations against *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* sp. and *Pseudomonas* sp. is shown in Table 2 and Figure 9. The antimicrobial activity of *Cabomba demersum* was earlier tested by Su *et al*., [28]. Similarly Pip and Philip [29] reported alkaloids in *C. demersum*. Bacteriocidal activity of extracts of the aquatic plant *Lemna minor* has been reported by Stagenberg [30].

Table 2. Antimicrobial activity exhibited by *Cabomba* extract in varying concentrations against selected bacteria

Extract	Concentration (µg ml^{-1})	Zone of inhibition (cm)			
		Gram positive bacteria	Gram negative bacteria		
		S. aureus	*E. coli*	*Klebsiella* sp.	*Pseaudomonas* sp.
Acetone	800	08 ± 0.3	05 ± 0.2	05 ± 0.3	04 ± 0.2
	600	06 ± 0.2	04 ± 0.2	03 ± 0.2	04 ± 0.1
	400	05 ± 0.3	03 ± 0.3	03 ± 0.2	03 ± 0.4
	200	03 ± 0.1	01 ± 0.1	01 ± 0.3	02 ± 0.3
	100	01 ± 0.2	01 ± 0.2	01 ± 0.3	01 ± 0.3

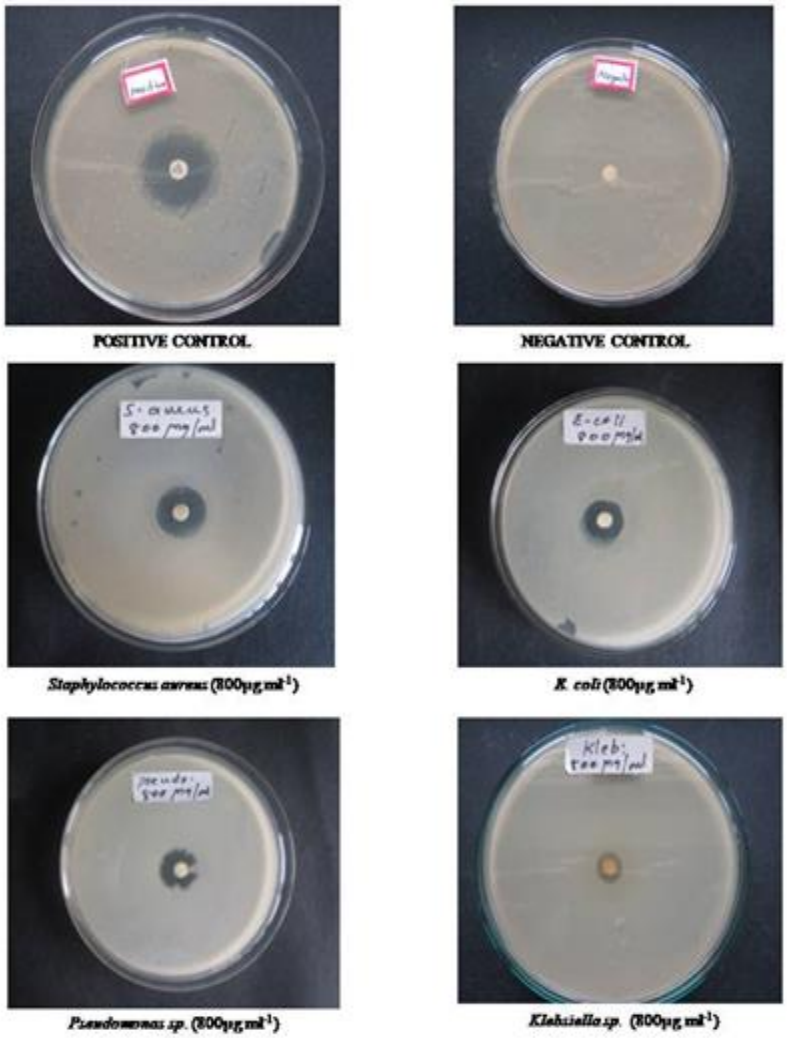

Figure 9. Antimicrobial effects of *Cabomba* extract (in acetone)

GC MS Analysis

The study on active principles of the acetone extract of *Cabomba caroliniana* by GC-MS showed the presence of thirteen compounds. The major and minor compounds with their Retention Times (RT), molecular formulae, Molecular Weights (MW) and peak area (%) are presented in Table 3. The major constituents were found to be Benzene-1,2-dicarboxylic acid (2.6%), 2,4-Bis (1-phenylethyl) phenol (2.3%), Gamma-Sitosterol (1.94%), 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester (1.5%). 0-(nitrobenzyl) hydroxylamine (1.3%) Gamma-Sitosterol has got antioxidant, antibacterial antidiabetic and prophylactic activities [31]. Pentadecanoic acid, 14-methyl-methyl ester is known to be a potential antimicrobial and antifungal agent [32]. Peak area percentage of this compound is 0.1. Another compound 3-Eicosyne having peak area percentage 0.0236 is also present. Studies conducted by Hsouna et al. [33] showed that Eicosyne has got antitumor, antibacterial, antifungal and cytotoxic effects. Dibutyl phthalate having peak area percentage 0.4310 is a potent antimicrobial, antiviral and antifungal agent [34]. Oleanoic acid and ursolic acid identified in *Cabomba furcata* [35] could not be detected during the present work.

Table 3. Major and minor compounds with their Retention Times (RT), molecular formulae, Molecular Weights (MW) and peak area (%)

S. No	RT (min)	Molecular Formula	Molecular Weight	Name of Compound	Peak Area%
1	57.028	$C_{29}H_{50}O$	414.7067	Gamma-Sitosterol	1.94
2	28.483	$C_{17}H_{34}O_2$	270.4507	Pentadecanoic acid, 14-methyl	0.0884
3	26.331	$C_{20}H_{38}$	278.5157	3-Eicosyne	0.0236
4	32.757	$C_{19}H_{38}O_2$	298.5038	Heptadecanoic acid, 15-methyl	0.062
5	2.887	$C_6H_7O_4P$	174.0911	Phosphonic acid	0.9981
6	37.547	$C_{12}H_{14}O_4$	222.2372	1,2-Benzenedicarboxylic acid, monobutyl ester	0.5398
7	29.157	$C_{20}H_{30}O_4$	334.4498	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	1.512
8	32.242	$C_{16}H_{22}O_4$	278.3435	Dibutyl phthalate	0.431
9	2.769	C_4H_9F	76.1127	Propane, 2-fluoro-2-	0.4585
10	5.611	$C_7H_8N_2O_3$	168.15	0-(-4 nitrobenzyl) hydroxylamine	1.326
11	37.721	$C_{22}H_{22}O_4$	302.4161	2,4-Bis(1-phenylethyl)phenol	2.341
12	6.83	$C_{18}H_{24}O_4$	304.3862	Benzene-1,2-dicarboxylic acid O2-butyl ester O1-cyclohexyl ester	2.631
13	36.862	$C_{18}H_{24}N_2$	268.402	1,4-Benzenediamine, N-(1,3-dimethylbutyl)-N'-phenyl	0.3016

Michael and Nicholas [36] observed the pigments chllorophyll, carotenoids in submerged angiosperms which varied in wide range due to ecological conditions such as light and temperature. Aquatic plants are proving to be an increasingly valuable reservoir of compounds and extracts of substantial medicinal merit. A triterpenoid was reported [37] from an aquatic herb *Nymphoides cristatum*, which was used for treatment of fever and jaundice. The crude extract of *Trapa bispinosa* possesses the antimicrobial and cytotoxic acitivty; few compounds were reported from the extract of *T. bispinosa* exhibiting broad spectrum antibacterial properties [38]. Malathy and Shaleesha [39] reported antibacterial and antifungal activity of crude extracts of *Cabomba aquatic.*

CONCLUSION

The present study on extraction, isolation and characterization of bioactive compounds from the aquatic weed *Cabomba caroliniana* A. Gray was carried out to evaluate the antiarthritic, antioxidant, antiinflammatory activities of the plant. From the present work, it could be concluded that the plant is endowed with many potent phytochemicals. Therefore the potent possibilities of this plant which possess high therapeutic value needs to be fully utilized. This would serve the dual purpose of preventing the water weeds from obliterating the aquatic ecosystems and producing useful compounds.

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REFERENCES

1. H Ozbay; A Alim. *Molecules*. **2009**, 14, 321-328.
2. JL Morales; ZO Cantillo-Ciau; I Sanchez-Molina; Mena-Rejon. *Pharm. Boil.* **2006**, 44, 632-635.
3. JA Okojie. Utilization of aquatic & wetland resources, Proceedings of 14th & 15th Annual Conference on of Aquatic resources Society, **1998**.
4. RMS Martins; AK Ikeda; MH Lourenco; LC Santos; EA Silva; NM Rocha. IV Simpósio Sobre Recursos naturais E Sócio-Econômicos Do pantanal, Corumbä/Ms-23-26 Nov. **2004.**
5. G Wollf; LR Assis; GC Periera; JG Carvalho; EM Castro. *Planta Daninha*. **2008**, 26, 315-325.
6. K Biswas; CC Calder. Handbook of Common Water and Marsh Plant of India and Burma, New Delhi **1936**.
7. CDK Cook. Aquatic and Wetland Plants of India, Oxford Univeristy Press, Oxford, U.S.A., **1996.**
8. K Subramanyan. Aquatic angiosperms, Council of Scientific and Industrial Research, New Delhi. **1962.**
9. OO Oyedapo; BA Akinpelu; M Akinwunmi; MO Adeyinka; O Sipeolu. *International Journal of plant Physiology and Biochemistry.* **2010**, 2(4), 46-51.
10. J Sadique; WA Al-Rqobahs; Ar Bughaith El-Gindi. *Fitoteralia*. **1989**, 60, 525-532.
11. KH Kim; YW Kim; HB Kim; BJ Lee; DS Lee. *Biotechnology Letters.* **2006**, 28, 439-446.
12. YA Chabbert. *Bot Mar*, **1963**, 31, 375.
13. A Nostro; MP Germano; A Angelo; VD Marino; MA Cannatelli. *Lett App Microbio*. **2000**, 30, 379-384.
14. CD Preston; JM Croft. Aquatic plants in Britain and Ireland. Botanic Survey of British society of the British Isles (BSBI), Colchester. **1997**, 70-312.
15. D Moerman. Native American Ethnobotany. Timber Press, Portland, Oregon, USA,**1998**, 42-691.
16. JF Hu; E Gaaro; MG Goering; M Pasmore HD Yoo; T Esser; J Sestrich; PA Cremin; GH Hough; P Perrone, YSL Lee, NT Le; M O'Neil-Johnson; JW Costerton; GR Eldridge. *J Nat Prod*, **2006**, 69, 118-120.
17. DA Vattem; K Mihalik; SH Crixell; RJ McLean. *Fitoterapia.* **2007**, 96, 483-487.
18. S Shenoy; K Shwetha; K Prabhu; R Maradi; KL Bairy; T Shanbhag. Asia Pac J for Med, **2010**, 3(3), 193-195.
19. OI Aruoma; B Halliwell. *Biochemical Journal.* **1987**, 241(1), 273-278.
20. JR Soareset; TCP Dinis; AP Cunha; LM Ameida. *Free Radical Research.* **1997**, 26(5), 469-478.
21. C Evans; J Sampson; PM Bramley; DE Holloway. *Free Radical Research*, **1997**, 26, 381-389.

22. M Oktay; I Gulein; I Kufrevioglu. *Labenson-Wiss V Technol*. **2003**, 36, 263-271.
23. JL Reichelt; MA Borowitzka. *Hydrobiologia*, 1984, 22, 158-167.
24. AS Manilal; S Sujith; J Selvin; GS Kiran; C Shakir; CR Gandhimathi; MVN Panikkar. *J Mar Sci Tech*. **2009**, 17, 67-73.
25. C Premkumar; P Santhanam; J Sivakumar. *Seaweed Res Utiln*. **2013**, 83-89.
26. N Dhayanithi N; TT Ajith Kumar; R Ganesha Murthy; K Kathiresan. *Asian Pacific Journal of Tropical Biomedicine*. 2(3), **2012**, S1892-S1895.
27. GG Mary; O Sanaa; A Tarek. *Egyptian Journal of Natural Toxins*. **2007**, 4(2), 12-25.
28. KL Su; Abdul Haji; *EJ Staba Lioyda*. **1973**, 36, 80-87.
29. E Pip; K Philipp. *Hydrobiol*. **1990**, 75, 71-78.
30. M Stagenberg. *Hydrobiologia*. **1968**, 32, 88-96.
31. B Venkata Raman; LA Samuel; M Pardha Saradhi; B Narashinha Rao; A Nagavamsi Krishna; M Sudhakar; TM Radhakrishnan. *Asian Journal of Pharmaceutical and Clinical research.* **2012**, 5(2).
32. A Bashir; K Ibrar; B Shumaila; Sadiq Azam. *Journal of Medicinal plants Research*. **2012**, 6(31), 4653-4659.
33. AB Hsouna; M Trigie; RB Mansour; RM Jarraya, M Damak; S Jaoua. *International Journal of Food Microbiology*. **2011**, 148(1), 66-72.
34. K Elija; B Vaishali; Adsul; M Manik; NR Kulkarni; RV Kashalkar. *International Journal of Chem Tech Res* **2012**, 2(3), 1698-1701.
35. Z Omar; N Saadon; MNS Salleh; M Norzila. Business Engineering and Industrial Application Colloquium (BEIAC), **2013**.
36. PC Michael; AW Nicholas. *Plant Systematics, Evolution*, **1998**, 210, 105-111.
37. A Rahman; AI Abu Sayeed; D Chowdhary; G Sadik; GRM Astaq Moha Khan. *J Biol Sci*. **2002**, 2, 46-48.
38. MM Rahman; Mosaddik; MII Wahed; ME Haque. *Fitoterapia*. **2000**, 71, 704-706.
39. R Malathy; AS Shaleesha. *Journal of chemical and Pharmaceutical research*. **2015**, 7(4), 479-483.