

A Review: Aquatic Macrophyte *Ceratophyllum demersum* L. (Ceratophyllaceae): Plant Profile, Phytochemistry and Medicinal Properties

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Abstract: *Ceratophyllum demersum* (CD) (Ceratophyllaceae) is one of the important and fascinating aquatic macrophyte that has a little recognition in traditional system of medicine for the treatment of various life threatening diseases and other areas in which its use can be utilized. There is less knowledge available about phytoconstituents and medicinal properties of the plant. In other words, compiled information about its phytoconstituents, medicinal properties and other related information is lacking till date. In the present review, the author tried to compile all the phytoconstituents, medicinal and other related information of CD and fill the knowledge lacuna in this particular field and encourages further study on the plant and other aquatic macrophytes as these definitely will help in developing potential biopharmaceutical product.

Keywords: *Ceratophyllum demersum*, aquatic macrophyte, phytoconstituents, medicinal properties

1. Introduction

Aquatic macrophytes are water vegetation comprising macroalgae and the true angiosperms. They form the bases of aquatic ecosystems and play fundamental roles in nutrient cycling of water bodies and provide suitable habitats for many other animals. They also determine the physico-chemical properties and nutrient cycling of water bodies. Aquatic macrophytes may be classified as emergent (e.g. cattails), free-floating (e.g. water lilies), or submerged macrophytes (e.g. coontail) [1, 2, 3].

Plant Profile



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Domain: Eukaryota
Kingdom: Plantae
Division: Spermatophyta
Sub-division: Angiospermae
Class: Dicotyledonae
Order: Nymphaeales
Family: Ceratophyllum
Species: demersum

Ceratophyllum demersum (CD) also known as coontail or hornwort is a dicotyledonous, submerged aquatic angiosperm belonging to the family Ceratophyllaceae. This family comprises three species: *C. demersum*, *C. submersum* and *C. muricatum*. *C. demersum* occasionally branches but with a single branch produced per node. Leaves are mid-dark green, rigid sessile, in whorls of 6-8, dichotomously divided (1- or 2-3-forked) into linear segments with 4 or 5 prominent teeth marginally. Roots are lacking, but leafy branches are sometimes modified as rhizoids; stems break easily and the pieces continue growth separately. Flowers are unisexual; both staminate and pistillate on the same plant; very small, solitary in axil of one leaf of a given whorl, each subtended by an 8-12-part involucre; they have no perianth. The staminate flowers have 4-10 stamens, with very short filaments, anthers with a connective projecting distally and ending in 2 bristles. Pistillate flowers have 1 pistil and a superior, 1-locular, ovary. The fruit is 1-seeded, ovoid-oblong 4-6mm long achene, with spineless, lateral margins and 1 or 2 basal spines [4]. It exhibits a cosmopolitan distribution and has a wide ecological tolerance [5]. CD occurs in quiet or slow flowing, hard calcareous nutrient-rich or eutrophic waters of streams, ditches, canals, ponds and lakes as a near free-floating aquatic plant where it may form large masses [6,7]. It perennate in temperate regions by dormant apices on the lake bottom where these organs are covered by a layer of detritus. Dormancy is initiated in late summer and autumn when elongation growth of the lateral shoots ceases, and tightly clustered, dark-green leaves, which contain high levels of starch, are formed [8].

The natural enemies of *C. demersum* are *Ctenopharyngodonidella* (a predator) [9,10], *Hirschmanniella caudacrena* (parasite) [11] and *Mycoleptodiscus terrestris* (pathogen) [12]

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C. demersum has been widely used as bioindicators of heavy metals in air pollution, radioactivity indicators [13,14], biomonitoring [15] in the aquatic environment, genetic engineering [16]. It is also one of the popular plants in aquatic industry due to its tolerance in a wide range of aquatic conditions [17,18]. It also provides an excellent living environment for shelter to fish and aquatic organisms. It is also used as a source of food for some livestock, poultry [19] and fish [20]. *C. demersum* is astringent, bitter, sweet, oleaginous, fragrant and purgative [21]

2. Material and Methods

In the present review, information about *Ceratophyllum demersum* and its phytochemical constituents, medicinal properties and biochemical properties was gathered via searching scientific databases including PubMed, Elsevier, Google Scholar, Springer etc. and other related books either online or offline.

Aim of Present Review

Information about medicinal properties of *C. demersum* L. and its phytoconstituents is segregated and not presented in well documented form. In the present review, information has been compiled regarding phytochemistry, medicinal properties and other related information of *Ceratophyllum demersum* L.

PHYTOCHEMISTRY

C. demersum plant extracts consist of different classes of phytochemicals such as alkaloids, cardiac glycoside, glycosides, tannins and flavonoids [22,23]. The proximate composition (% of dry weight) of *C. demersum* includes dry matter (6.9%), crude protein (15.8), ash (25.3), crude fat (3.0), crude fibre (20.7), nitrogen free extract (35.2), calcium and magnesium [24, 25,26]. The essential oil from leaves of *C. demersum* was isolated by steam distillation in yield of 0.15% and its chemical composition was examined by GC and GC-MS. The main components present in the plant's essential oil include 2-methylpropanoic acid 3-hydroxy-2,4,4-trimethylpentyl ester (>15%), 2-methylpropanoic acid 2,2-dimethyl-1-(2-hydroxy-1-methylethyl)propyl ester (>3%), β -ionone-5,6-epoxide (>7%), toluene (>6%), hexanal (>5%) and 1,2-benzenedicarboxylic acid di(2-methylpropyl) ester (>5%) [27]. Tricin-7-O- β -D-glucoside, naringenin-7-O- β -D-glucoside, esculetin, β -sitosterol, 7 α -hydroxyl- β -sitosterol, 7 α -methoxyl- β -sitosterol and palmitic acid were also isolated from *C. demersum* [28]. In another study, two flavonoid glycosides were also isolated and one was identified as apigenin-7-O-glucoside. Seven sterols, the main one sitosterol, were also identified. Volatile compounds contained mainly n-paraffins, together with benzyl acetate and a sesquiterpene were isolated and identified [29]. Gentisic acid, vitamin C, coumaric acid, ferulic acid and beta-carotene were also detected in the extract of *C. demersum* in another study [30].

3. Medicinal Properties

Ethnomedicinal Properties

The ethnomedicinal uses of the plant *C. demersum* suggested vital role in the treatment of various diseases. Its juice mixed with sesamum oil is prescribed for discoloured

skin. 10-15ml decoction of the plant given twice daily for 7-10 days cures biliousness and ulceration [31,32]. In Indian medicine, the herb is used in jaundice, for scorpion stings, as an antipyretic and antimalarial. In China, it is used with hemoptysis. The plant is a cooling antiperiodic and also useful in the treatment of diarrhea, dysentery and constipation. It is carminative, styptic. The whole plant has been traditionally used in the treatment of wounds, fever, burning sensation, haemorrhoids or piles, intrinsic haemorrhages, hyperdipsia, epistaxis, haematemesis [21, 33]. It is prescribed for cardiac infections, giddiness, haemothermia, leucorrhoea, morbid thirst, rheumatism, spermaturia, venereal diseases [32,34]. Leaf juice is used to stop vomiting, as cooling agent and to cure skin disorders [35, 36].

Pharmacological Properties

A long history of *C. demersum* has led modern day researcher to study the various extracts and its significant pharmacological activities. Its traditional use against dysentery, pyretic and wounds has been validated in various studies.

Antidiarrhoeal and wound healing activity

The methanol and aqueous extracts of whole plant of *Ceratophyllum demersum* were assessed for antidiarrhoeal and wound healing potentials using experimental models in rats. Acute oral toxicity studies were performed according to the OECD 423 guidelines. All the doses of (5, 50, 300, 2000 and 5000 mg/kg) of methanol and aqueous extracts employed for acute oral toxicity studies were found to be non-toxic. Both the extracts did not produce any mortality even at the highest dose (5000 mg/kg). Antidiarrhoeal activity at a dose of 250 and 500 mg/kg was evaluated in castor oil and magnesium sulphate induced diarrhoea in rats and the parameters studied were: total no. of faeces, no. of wet faeces, percentage inhibition of defecation and diarrhoeic drops. Wound healing activity of the both the extracts (5 % w/w ointment in simple ointment base) was evaluated in excision wound model and the parameters taken into account were percentage of wound closure and epithelialization time. Both the extracts at a dose of 500 mg/kg showed significant antidiarrhoeal and also showed significant wound healing activities (5 % w/w) [22].

Antioxidant and anti-acetylcholinesterase (AChE) activity

The ethanol extracts from *Ceratophyllum demersum* L. was assessed with other aquatic plants *in vitro* for their antioxidant and anti-acetylcholinesterase (AChE) activities. Antioxidant activity was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity test at 0.125, 0.25, 0.5, 1.0, and 2.0 mg/ml and ferric-reducing antioxidant power assay (FRAP) at 0.5, 1.0, and 2.0 mg/ml concentrations. Total phenolic contents (TPC) of the extracts were determined using Folin-Ciocalteu's reagent. The TPC of the extract was 528.29 \pm 4.07 and percentage of inhibition against DPPH radical include; at 0.5, 1.0 and 2.0 mg/ml concentration, the values were 12.2 \pm 1.06, 22.6 \pm 0.62 and 39.3 \pm 1.11 as against the reference using Gallic acid (GA) and BHA at the same concentration with values of GA (91.6 \pm 0.06, 92.6 \pm 0.10 and 93.2 \pm 0.00) and BHA (77.9 \pm 0.48, 81.6 \pm 1.67 and 82.9 \pm 0.66). In FRAP, at 0.5 and 1.0 mg/ml,

the values were 0.329 ± 0.02 and 0.609 ± 0.02 against GA and BHA at 0.25 and 0.50 mg/ml with values of GA (3.569 ± 0.02 and 3.677 ± 0.02) and BHA (1.47 ± 0.01 and 2.595 ± 0.44). In the anti-AChE assay, the extract exerted insignificant inhibition against the enzyme at 1.0 mg/ml and 2.0 mg/ml concentrations which had percentage inhibition of 7.1 ± 1.92 and 11.2 ± 2.81 with reference drug (Galanthamine) which had 98.9 ± 0.24 [29].

In vitro free radical scavenging activities of aqueous and methanol extracts from *in vitro* propagated *C. demersum* using DPPH were investigated. The extracts from *C. demersum* were effective in the following order: water > methanol. DPPH scavenging activities of treatments with maximum concentration (10 mg/ml) of water and methanol extracts were 68.91 and 51.22% respectively and IC_{50} values of water and methanol extracts showed aqueous as most effective extract with 3.689 mg/ml and methanol extract having 10.301 mg/kg. gallic acid (standard drug) was computed as 0.029 mg/ml with statistically ($p < 0.05$) significant difference between this value and the values belonged to extracts [37].

In another study, the response of *C. demersum* when exposed to 1,2- and 1,4-dichlorobenzene (1,2-DCB and 1,4-DCB) through the assay of glutathione reductase (GR), guaiacol peroxidase (POD) and glutathione peroxidase (GPx) was evaluated. Additionally, the effect of DCBs on the detoxication system by measuring the activity of glutathione-S-transferase (GST) was also evaluated. The plants showed elevated GST activities when exposed to 10 and 20 mg/l 1,2-DCB, and at 10 mg/l for 1,4-DCB. The results showed that glutathione conjugation take place at relatively high concentrations of both isomers. Significantly increased activities of POD were also detected in *C. demersum* exposed to concentrations above 5 mg/l of the corresponding isomer. The GR activity was enhanced in plants exposed to 1,2-DCB (5 mg/l) and 1,4-DCB (10 mg/l). GPx was also significantly increased in exposures to the corresponding isomer, each at a concentration of 10 mg/l. However, plants exposed to low doses of 1,4-DCB (1 mg/l) showed significantly decreased activities of both enzymes GR and GPx. Consequently, this study showed that the exposure of the aquatic macrophyte *C. demersum* to DCBs was able to cause an activation of the antioxidant system, showing an isomer specific pattern, which suggests that the defence system of the plant is playing an important role in scavenging ROS, helping to protect the organism against adverse oxidative effects generated by the prooxidant action of the tested xenobiotics [38].

Antimicrobial activity

The antimicrobial activity of *Ceratophyllum demersum* with other macrophytes were extracted with three different solvents like acetone, butanol and methanol and determined the activity against two pathogenic organisms both Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacteria and fungi (*Aspergillus niger*) by agar well diffusion method. Antifungal property was found in 50% acetone extract causing inhibition zone 18mm of *C. demersum* against *Aspergillus niger*. There was no activity recorded for the plant extract against *Staphylococcus aureus* and *Escherichia coli* [23]. In another study, the

antimicrobial activities of aqueous and organic solvents (chloroform, ethanol and methanol) extracts of *Ceratophyllum demersum* L., with other plants were tested *in vitro* against seventeen different microorganisms including Gram-positive and Gram-negative bacteria and fungi. Nine of these identified organisms were obtained from different sources, *Bacillus subtilis* 1020, *Bacillus cereus* 1080, *Staphylococcus aureus*, *Erwinia carotovora* NCPPB 312, *Candida albicans*, *Candida tropicalis*, *Aspergillus niger*, *Fusarium oxysporum* and *Penicillium italicum*. The other eight organisms were isolated from another source and identified using API 20E strip system (BioMereux). One hundred pathogenic bacteria isolates representing eight genera were identified to species level. These organisms are *Escherichia coli* (20%), *Pseudomonas aeruginosa* (16%), *Klebsiella pneumoniae* (14%), *Salmonella choleraesuis* (13%), *Shigella sp.* (11%), *Serratia liquefaciens* (10%), *Proteus vulgaris* (9%) and *Brenneria nigrifluens* (7%). The extract demonstrated antimicrobial activity against the used organisms. The efficiency of the extracts varied with, solvent used in the extraction. The aqueous extract appeared to be the highly effective extract against all tested organisms especially *Fusarium oxysporum* causing inhibition zone 48 ± 0.01 mm, *Pseudomonas aeruginosa* 59 ± 0.02 mm and *Salmonella choleraesuis* 55 ± 0.01 mm when using *C. demersum*. Ethanol extract of *C. demersum* showed antimicrobial activities against all tested organisms except *Aspergillus niger*. On using chloroform extracts *Escherichia coli*, *Aspergillus niger* and *Penicillium italicum* showed resistance [26].

Antineoplastic and anti-inflammatory activity

The assessment of the spectrum of biological activities (antineoplastic and anti-inflammatory) with prediction of activity spectra for substances (PASS) for the major components of essential oil of *C. demersum* along with other plants extracted with hexane was studied. The predicted value of anti-inflammatory and antineoplastic activities with probability above 0.8 was observed for 12 compounds (2Z,4Z)-Hepta-2,4-dienal; 2-Phenylacetaldehyde; (3E,5E)-Octa-3,5-dien-2-one; 2,6-Dimethylcyclohexan-1-ol; geranylacetone; α -muurolene; β -ionone; β -eudesmol; α -eudesmol; biformen; kaurene and manool [39].

The Anti-inflammatory activity of methanol extract of whole plant of *C. demersum* was also tested using the carrageenan induced rat paw edema model at the doses of 250 and 500 mg/kg with 1% CMC (10 mg/kg p.o) as control and Nimesulide (50 mg/kg p.o). The methanol extract of *C. demersum* at the dose of 250 and 500 mg/kg body weight showed significantly ($p < 0.01$) reduction paw volume [39].

Analgesic and antipyretic activity

Acetic acid induced writhing model was employed to evaluate the analgesic activity. Albino mice 2025 gm body weights were divided into four groups of six animals each. First group of the animals received 1% CMC (10 ml/kg of b.w., p.o) served as control, second group served as reference standard received Nimesulide (50 mg/kg of b.w., p.o) while third and fourth group received methanol extract (250 and 500 mg/kg of b.w., p.o), respectively. The vehicle, extract and standard drug administered orally 1 h. prior to the intraperitoneal

administration of acetic acid injection (10 ml/kg of 0.6% v/v). The writhing effect indicated by the contraction of abdomen with simultaneous extension of hind limbs and trunk twist response. The analgesic activity was expressed in term of percentage inhibition of writhes produced by acetic acid was calculated by using the formula, Percentage inhibition of writhes = $\frac{\text{Mean of Control} - \text{Mean of Test}}{\text{Mean of Control}} \times 100$.

The antipyretic activity was evaluated using Brewer's yeast (*Saccharomyces cerevisiae*) induced pyrexia method in Wistar rats. Before the experiment, the rats were maintained in separate cages with food and water ad libitum for 7 days and the animals with approximately constant rectal temperature (37.5–38.40) were selected for the study. Male Wistar albino rats weighing, 150–200 gm were divided into four groups of six animals each. First group of the animals received 1% CMC (10 ml/kg of b.w., p.o.) served as control, second group served as reference standard received Paracetamol (50 mg/kg of b.w., p.o.) while third and fourth group received methanol extract (250 and 500 mg/kg b.w., p.o.), respectively. Fever was induced by injecting 2 ml/kg of 20% aqueous suspension of Brewer's yeast in distilled water and 18 h after yeast injection the vehicle, extract and standard drug were administered. Rectal temperature was recorded by clinical thermometer at 0, 1, 2, 3 h after drug administration. The methanol extract of *C. demersum* (MECD) at the dose of 250 and 500 mg/kg body weight showed significantly ($p < 0.01$) reduction in number of writhes and paw volume and at the dose 500 mg/kg body weight showed significantly ($p < 0.05$) reduction in pyrexia [40].

Allelopathic and genotoxic activity

The genotoxic potential of aqueous extract of *C. demersum* was evaluated along with other aquatic plants using the micronucleus test and to find possible correlation with the total polyphenol and tannin content. For genotoxicity assessment, the mussel micronucleus test was applied. Micronucleus frequency was determined from the haemolymph of *Unio pictorum* L. (painter's mussel). In parallel, total and hydrolysable tannin contents were determined. The plant extract elucidated significant mutagenic effect. Significant correlation was determined between tannin content and mutagenic capacity. The significant correlation between genotoxicity as expressed by micronucleus frequency and tannin content (both total and hydrolysable tannins) indicate that tannin is amongst the main compounds being responsible for the genotoxic potential which might suggest the genotoxic capacity of the plant to elucidate a real ecological effect in the ecosystem [41].

The allelopathic activity of *Ceratophyllum demersum* was also evaluated and a bioassay-directed method development revealed optimal extraction solvents for allelochemicals from the plant. Extracts of *Ceratophyllum demersum* inhibited both *Anabaena* PCC 7120 and *Synechococcus elongatus* at all tested concentrations and with all solvent mixtures used. *Ceratophyllum* 50% acetone yielded the strongest inhibition in the agar-diffusion assay with various filamentous or chroococcal cyanobacteria as target species. Further fractionation by liquid-liquid extraction (LLE) and solid

phase extraction (SPE) procedures showed that the aquatic macrophyte appear to have more than one active fraction, one being hydrophilic and one moderately lipophilic. The water-soluble allelochemicals may inhibit phytoplankton whereas the lipophilic allelochemicals may act through direct cell-cell contact, e.g., against epiphytes. The macrophyte also exuded allelopathically active compounds into the surrounding medium as shown by SPE of their incubation water [42].

In another study, the potential relationships between the phytochemical compounds detected in the plant extract (acids, phenols, heterocycles, alcohols, hydrocarbons) and the allelopathic properties against Gram positive and Gram negative bacterial strains and microalgal cultures were investigated. The untargeted solid phase micro extraction (SPME) GC/MS analysis performed on the dried plants incubated for 25 min at 50°C showed that phenols are the major constituents of the volatile fingerprint with a relative peak area higher than 13%, namely: 2,6 bis(1,1-dimethylethyl)-4(1-oxopropyl) phenol and phenol, 3,5-bis(1,1-dimethylethyl). The inhibition effects *ex situ* of the analyzed extracts suggested that the aquatic macrophyte may excrete inhibitory substances and show allelopathic inhibitory potential against certain bacterial strains and on *Rhizoclonium hieroglyphicum* and *Microcystis aeruginosa* cultures [43].

4. Conclusion

Natural products have been an important resource for the maintenance of life since ages and are becoming increasingly important as alternative medicine. *C. demersum* had a long history of traditional uses for wide range of diseases. *C. demersum* plant studied in this review article is important as alternate for various disease research and treatment. This plant and their folkloric/traditional pharmacological profile need to be preserved. In recent years, it has been experimentally proved that this plant possess a number of biological activities. However, scientific validation of the plant as a modern medicine is lacking. Further studies need to be carried out to explore its potential and other aquatic macrophytes in the treatment of diseases by using isolated compounds with minimal or no side effects. More so, ensuring the safe use of these plants should be strived more. This plant needs effective utilization in order to make a hallmark through complete diseases cure and cheap regimen to be available for ordinary population vis-à-vis helping in developing potential biopharmaceutical product.

5. Disclosure/Conflict of Interest Statement

The author of this paper has no financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper. It is specifically stated that "No Competing interests are at stake and there is No Conflict of Interest" with other people or organizations that could inappropriately influence or bias the content of the paper. The manuscript has not been published previously by any of the authors and/or is not under consideration for publication in another journal at the time of submission.

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