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In-Vitro antioxidant activity of phyto-pharmacological and gc-ms analysis of bioactive compounds presents in *Eichhornia Crassipes* leaves ethanolic extract

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Abstract
Plants are rich source of the traditional medicine water hyacinths pose serious challenges to humanity and the environment. Consider the extent of the menace with the growth and spread of the plant and the difficulty in achieve a single, normally acceptable control method, it is attractive increasingly essential to explore the potentials of the plant. Phytochemical presence of Tannin, Saponin, Flavonoids, Steroids, Alkaloids and Polyphenol. GC-MS analysis of bioactive mixtures major Twelve bioactive compounds parts are distinguished as Retention time [8.95] Cyclohexasiloxane, dodecamethyl, biological activity of Emollient, in personal care products, lubricant and de-foaming agent. Hexadecanoic acid, methyl ester R.Time [15.97], Tetracosamethyl-cyclododecasiloxane R.Time [24.24], 9,12,15 Octadecatrienoic Acid R.Time [30.24]. *In-vitro* anti-oxidant activity of (A) DPPH scavenging activity of 12.5(μM) - Maximum value 1.42±0.71 and 100(μM) minimum value 19.76±1.27 IC_{50} (μM) ±SEM 35.13±1.79 (B) H_2O_2 scavenging activity of Maximum value 12.5(μM) 2.53±0.52 and 100(μM) minimum value 23.63±1.68 IC_{50} (μM) ±SEM 47.87±3.07 (C) Nitric oxide scavenging activity of Maximum value 12.5(μM) 3.82±0.64 and 100(μM) minimum value 24.41±2.16 IC_{50} (μM) ±SEM 39.31±4.87 (D) FRAP assay of maximum value of 12.5(μM) 2.58±0.58 and minimum value 100(μM) 20.41±1.83 IC_{50} (μM) ±SEM 38.85±3.85. *In-vitro* anti-oxidant activity of treated with high potential plant growth *Eichhornia Crassipes.*

Keywords: phytochemical, gc-ms, *in-vitro* antioxidant, dpph, h_2o_2, cyclohexasiloxane,

Introduction

The freshwater aquatic plant *E. crassipes*, ordinarily known as water hyacinth is an individual from the family *Pontederiaceae*. This quickly developing, free-coasting, lasting plant is native to Brazil Amazon bowl and the Ecuador locale. It was acquainted as an elaborate species with decorate the water bodies. This intrusive weed represents various risks going from natural and efficient to social [1]. It will in general jeopardize biodiversity, cause eutrophication, cover bugs, stop up new streams, influence farming and hydroponics and hamper transportation and sporting exercises. Existing control techniques have been deficient to contain its forceful spread. A considerable lot of the bird asylums and water bodies have been definitely attacked by this amphibian weed which is influencing environmental specialty and bird movements. A few analysts have effectively shown the utilization of water hyacinth in bio-remediation and as a possible wellspring of sustainable power [2]. Distinctive substantial and poisonous metals showing biomagnifications and consequently making wellbeing dangers could be remediated by utilizing water hyacinth. The way toward sterilizing agro-mechanical waste dirtied with hefty metals, natural and inorganic toxins should be possible utilizing water hyacinth. The current audit centres on the endeavours to use this weed for various worth added and restorative properties. Besides, the article stresses the need to acquire knowledge into the instrument with concrete randomized controlled examinations to discover the impacts of this weed on human wellbeing and an economical answer for misuse and deal with this obtrusive in any case destructive weed into a valuable substance for humankind [3]. A large number of the bird safe-havens and water bodies have been definitely attacked by this amphibian weed which is influencing biological specialty and bird movements. Phyto-remediation through sea-going macrophytes treatment framework (AMATS) for the expulsion of poisons and pollutants from different regular sources is a grounded ecological assurance strategy [4]. Water hyacinth (*Eichhornia crassipes*), the most exceedingly terrible intrusive oceanic weed has been used for different examination exercises throughout the most recent couple of many years. The biosorption limit of the water hyacinth in limiting different impurities present in mechanical wastewater is all around examined [5]. Distinctive weighty and poisonous metals showing biomagnifications and consequently making wellbeing dangers could be remediated by utilizing water hyacinth. The way toward cleaning agro-mechanical waste dirtied with substantial metals, natural and inorganic contaminations should be possible utilizing water hyacinth. The current survey centres on the endeavours to use this weed for various worth added and restorative properties [6]. Besides, the article stresses the need to acquire understanding into the instrument with concrete randomized controlled examinations to discover the impacts of this weed on human wellbeing and a feasible answer for misuse and deal with this intrusive in any case hurtful weed into a

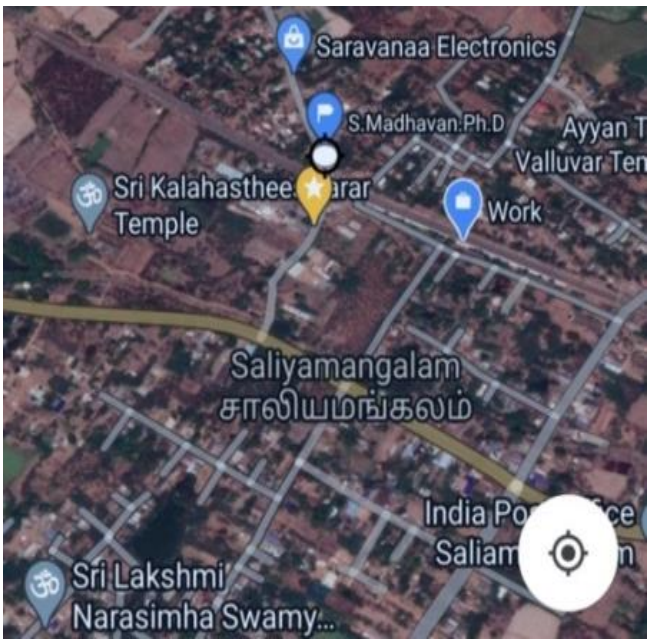
valuable element for humankind. The phytochemical structure of *E. crassipes* decided is summed up.

Materials and Methods

Plant Collection

The fresh leaves of *Eichhornia Crassipes* were collected from Saliyamangalam, Thanjavur District, Tamil Nadu, India.

Fig 1: *Eichhornia Crassipes Leaves*



Map 1: Study area

Phytochemical Studies

Secondary metabolites in the present studies were carried out on the plant sample revealed the presence of medicinally active constituents. Beneficial drugs and to improve the patient health.

Preparation of extracts

The powdered plant tests of leaf (100 g) were utilized for progressive dissolvable extraction (500ml) with expanding request of polarities like methanol and Aqueous. By then it is kept in an orbital shaker at 190-220rpm for 48 hours. The supernatant was gathered, sifted through Whatman No.1filter paper and the concentrate were concentrated by a Rotary carafe evaporator at a particular temperature was utilized dependent on the dissolvable framework. Each time prior to extricating with the following dissolvable the buildup was dried completely to eliminate the dissolvable utilized. They got dried concentrate was then precisely gauged, put away in little vials at - 20°C and utilized for the accompanying investigations.

Phytochemical screening

The preliminary phytochemical evaluation was carried out by using standard procedure [7-11].

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

Clarus 500 Perkin- Elmer (Auto System XL) Gas Chromatograph equipped and coupled to a mass detector Turbo mass gold – Perking Elmer Turbomas 5.2 spectrometer with an Elite-1 (100% Dimethyl ply siloxane), 300 m x 0.25 mm x 1 µm df capillary column was used for GCMS analysis. Initially, the instrument was set to temperature of 110°C, and then maintained at the same temperature for 2 min. At the end of this period, the oven temperature was raised upto 280°C, at the rate of an increase of 5°C per minute and maintained for 9 min. The temperature of the injection port was ensured as 250°C and the flow rate of Helium as 1 ml/min. The ionization voltage was 70 eV. The samples were injected gradually in split mode as 10:1. The range of mass spectrum was set at 45-450 (mhz). The chemical constituents were identified by GC-MS. The discontinuity examples of mass spectra were contrasted and those put away in the spectrometer information base utilizing National Institute of Standards and Technology Mass Spectral information base (NIST-MS). The percentage of each component was calculated from relative peak area of each component in the chromatogram.

Identification of Compounds

Translation of a mass range of GC-MS was directed utilizing the information base of the National Institute Standard and Technology (NIST) having more than 62,000 examples. The unknown component's spectrum was compared with the spectrum of the known components stored in the NIST library. The structure, name and sub-atomic load of the parts of the test materials was learned.

In-vitro anti-oxidant assays

DPPH free radical scavenging assay

The DPPH radical-scavenging activity of the test extracts was examined using the modified method by Brand-Williams *et al*. [12]. Leaf extracts of different concentrations

(50e200 mg/mL) were mixed with an equal volume of Methanolic solution of DPPH (Sigma Aldrich). The mixture was allowed to react at room temperature in dark for 30 min. Ascorbic acid (1 mg/mL (50e200 mg/mL)) was used as positive control. After 30 min the absorbance was measured at 517 nm and converted into a percentage of antioxidant activity using the following equation.
% of inhibition = [A0-A1/A0] *100
Where A0 =Absorbance of control.
A1 = Absorbance of the test.

Hydrogen peroxide scavenging assay
The H_2O_2 scavenging activities for both the leaf extracts were assayed by the modified method [13]. A different concentration of plant leaf extracts (50e200 mg/mL) and ascorbic acid at different concentrations (50e200 mg/mL) of (1 mg/mL) were added to 40 mM H_2O_2solution prepared in phosphate buffer. The absorbance of H_2O_2 at 230 nm was determined after 10 min. The percentage of H_2O_2 scavenging by the extracts and standard (H_2O_2) was calculated as follows.
% of scavenged [H_2O_2] = [A0-A1/A0] *100
Where A0 =Absorbance of control.
A1 = Absorbance of test

Nitric oxide radical scavenging assay
The nitric oxide (NO) scavenging activity was determined using the method described by Parul *et al.* [14]. 10 mM sodium nitroprusside was incubated with 100 mL leaf extract for 60 min at 30 _C. After incubation, 100 mL of Griess reagent was added. The absorbance of the chromatophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylehylendiamine was measured at 562 nm. Ascorbic acid (1 mg/mL) was at the same concentration was taken as standard.

% NO scavenged = [A0-A1/A0] *100
Where A0 =Absorbance of control.
A1 =Absorbance of test.

Statistical analysis
All assays were performed in triplicate. Mean and standard deviation (SD) was examined for all assays. The results were expressed as mean ± SEM of three experiments. One way ANOVA with Dunnett's test was followed to compare each concentration with a positive control to analyze the level of statistical significance. $P < 0.05$ were considered statistically significant using Graph pad PRISM v.8.0.

Results and Discussion
Phytochemical analysis of Presences and absences *E. crassipes* revealed the presence of secondary metabolites of anticancerous, antimicrobial, antioxidant, antidandruff, antiproliferative activities and provides a potential source of industrial application [15]. We concluded that the biological values of *P. stratiotes* and *E. crassipes* contain pharmacological active compounds that may enhance its use as a traditional drug.

Table 1: Phytochemical Analysis of Ethanolic leaves extracts of *E. crassipes*

S. No	Analysed Phytochemicals factor	Ethanol	Methanol
1.	Tannin	+	+
2.	Saponin	+	-
3.	Flavonoids	+	+
4.	Steroids	++	-
5.	Alkaloids	+	-
6.	Polyphenol	++	+

Indications: "+" means positive activity, "-" means negative activity

This resolve work on essential appreciation of the unpleasant drug unmistakable verification plan important in traditional thought practices and could incite inverse pharmacology by which society would get regular meds at a sensible cost.

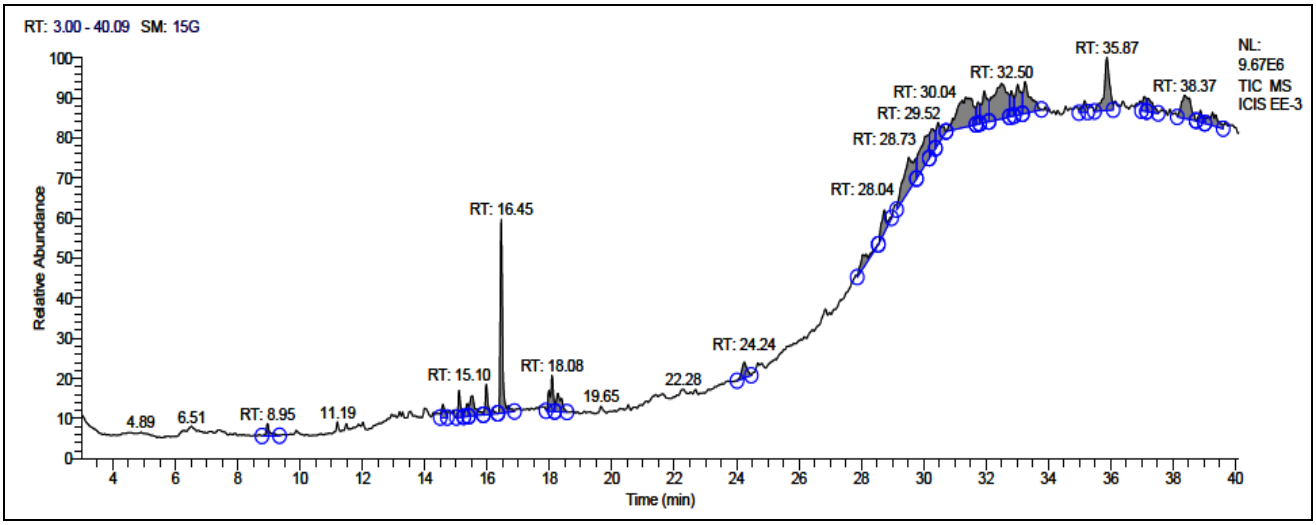


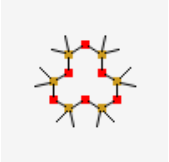
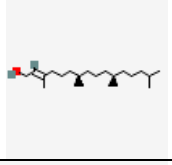
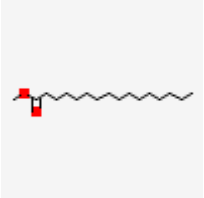
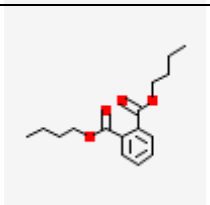
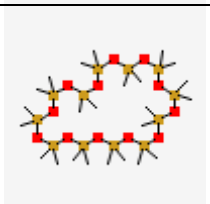
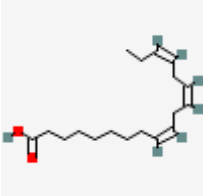
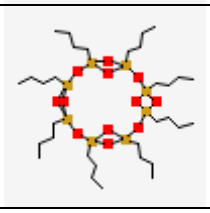
Fig 2: GC-MS Chromatogram of Ethanolic Extract *E. crassipes*

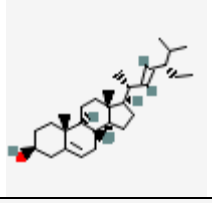
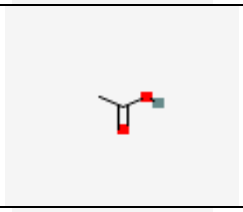
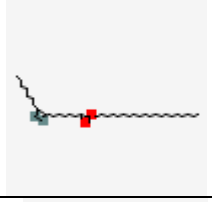
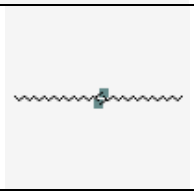
Table 2: GCMS analysis - Bioactive compounds

	Name of the Compounds	Retention time	Molecular Formula	Molecular weight g/mol
1	Cyclohexasiloxane, dodecamethyl	8.95	$C_{12}H_{36}O_6Si_6$	444
2	Phytol	15.10	$C_{20}H_{40}O$	296
3	Hexadecanoic acid, methyl ester	15.97	$C_{17}H_{34}O_2$	270

4	Dibutyl phthalate	16.45	$C_{16}H_{22}O_4$	278
5	Tetracosamethyl-cyclododecasiloxane	24.24	$C_{24}H_{72}O_{12}Si_{12}$	889
6	Hexasiloxane	29.52	O_5Si_6	248
7	9,12,15 Octadecatrienoic Acid	30.24	$C_{18}H_{30}O_2$	278
8	Octasiloxane	32.81	$C_{32}H_{72}O_{12}Si_8$	873
9	Stigmasterol	35.87	$C_{29}H_{48}O$	412
10	Acetic acid	37.19	$C_2H_4O_2$	60
11	Oleic acid, eicosyl ester	38.37	$C_{38}H_{74}O_2$	563
12	17-Pentatriacontene	38.37	$C_{35}H_{70}$	490

Table 3: GC-MS Analysis of Activities/Uses of bioactive compounds of

	Name of the Compounds	Structure	Activity / Uses
1	Cyclohexasiloxane, dodecamethyl		Emollient, in personal care products, lubricant and de-foaming agent.
2	Phytol		Antimicrobial, anti-inflammatory, diuretic, Anti-cancer,
3	Hexadecanoic acid, methyl ester		Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavour, Hemolytic, Alphareductase inhibitor
4	Dibutyl phthalate		anti-inflammatory and anti-apoptotic effects, Antitumor agent
5	Tetracosamethyl-cyclododecasiloxane		hepatoprotective activity, antispasmodic, antirheumatic, anti-soporific baths, insecticides for mosquito control, appetizing agent
6	Hexasiloxane		Antimicrobial, Antiseptic, Hair Conditioning Agent, Skin- Conditioning Agent-Emollient; Solvent
7	9,12,15 Octadecatrienoic Acid		Antiinflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Nematicide, Insectifuge Antihistaminic, Antiarthritic, Anticoronary, Antieczemic, Antiacne, 5-Alpha reductase inhibitor Antiandrogenic
8	Octasiloxane		Antimicrobial, Antioxidant

9	Stigmasterol		Antimicrobial, Anticancer, Diuretic, Anti-inflammatory, Antioxidant
10	Acetic acid		Anti-bacterial and Anti-fungal
11	Oleic acid, eicosyl ester		Insectifuge, antiinflammatory, cancer preventive and hypocholesterolemic
12	17-Pentatriacontene		Antiinflammatory Anticancer Antibacterial Antiarthritic

The GC-MS analysis of *E. crassipes* leaves revealed the presence of 11 major compounds n- Hexadecanoic acid (2.34%), E-11-Hexadecanoic acid, ethyl ester (1.04%), Palmitic acid, ethyl ester (12.09%), Phytol (2.12%), 9,12-Octadecadienoic acid, ethyl ester (3.79%), Linolenic acid, ethyl ester (26.26%), Stearic acid, ethyl ester (0.98%), Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (0.87%), α-Glyceryl linoleate (1.35%), 1-Monolinoleoylglycerol trimethylsilyl ether (1.52%), Stigmasterol (11.39%). The identified compounds possess many biological properties [16].

In-vitro anti-oxidant

Macrophage response to microbes is almost as fast as neutrophils, but macrophages live longer than neutrophils. Macrophage phagocytosis is also additional active in dealing with pathogens such as microorganisms or other antigens, and even cells or tissues themselves are damaged or dead so that macrophages can be considered as primary effector cells in the natural immune response (17).

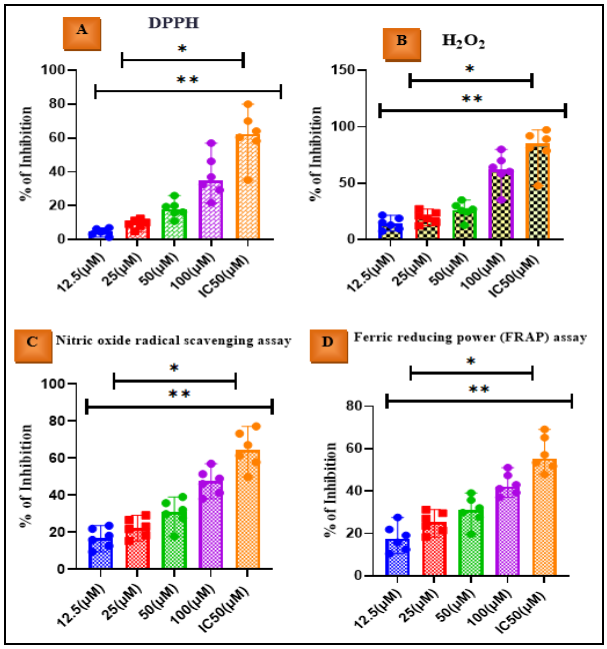


Fig 4: In-vitro antioxidant activity of ethanolic leaf extracts of *Eichhornia Crassipes* Leaves. (A) DPPH scavenging activity (B) H_2O_2 scavenging activity (C) Nitric oxide scavenging activity (D) FRAP assay. Values are expressed as Mean ± SEM (n = 3). One-way ANOVA followed by Dunnett's test was employed to compare each concentration with positive control. *Statistical significance at $p < 0.05$; ** statistical significance at $p < 0.01$. AsA-Ascorbic acid (Positive control).

Phytol is a deterrence compound and it might go about as an antimicrobial, calming, against malignancy and diuretic. Phytol was found to give great just as preventive and restorative outcomes against joint inflammation [18]. The outcomes show that receptive oxygen species advancing substances, for example, phytol comprise a promising novel class of drugs for the therapy of rheumatoid joint pain and perhaps other constant incendiary illnesses [19-21]. A significant number of the bird safe-havens and water bodies have been radically attacked by this sea-going weed which is influencing natural specialty and bird relocations. A few specialists have effectively exhibited the utilization of water hyacinth in bio-remediation and as an expected wellspring of sustainable power. of the plant exists. There are major methods for domineering the plants while control becomes inevitable: mechanical, chemical, and biological.

Summary and Conclusion

Rummaging of hydroxyl world-shattering is a important cancer prevention agent society as a result of high reactivity of the OH extremist which empower it to counter through a wide scope of particles found in living cells, like sugars, amino acids, lipids and nucleotides. Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. These exogenous antioxidants are normally called nutritional antioxidants. Cell reinforcements advantage the body by killing and eliminating the free revolutionaries from the circulatory system, Fruits, vegetables and grains are rich wellsprings of dietary antioxidants. A few specialists have effectively shown the utilization of water hyacinth in bio-remediation and as a possible wellspring of sustainable power. Distinctive substantial and poisonous metals showing bio magnifications and along these lines making wellbeing risks could be remediated by utilizing water hyacinth. The way toward disinfecting agro-modern waste dirtied with weighty metals, natural and inorganic poisons should be possible utilizing water hyacinth. The current survey centres on the endeavours to use this weed for various worth added and remedial properties. Besides, the article underlines the need to acquire understanding into the instrument with concrete randomized controlled investigations to discover the impacts of this weed on human wellbeing and a maintainable answer for abuse and deal with this obtrusive in any case hurtful weed into a useful substance for humankind. Oxidative pressure the result of an irregularity of supportive of oxidants and cancer prevention agents in the organic entity and is a critical wonder in ongoing illnesses. Oxidative pressure is currently recognized to be related with in excess of 100 sicknesses, just as with the ordinary maturing measure, particularly individuals determined to have Alzheimer's illness. Cell reinforcements are personally engaged with the avoidance of cell harm the normal pathway for malignancy, maturing, and an assortment of infections. A very good *in vitro* membrane stabilization effect and protein denaturation effect showed that the plant extract possessed anti-inflammatory and anti-arthritic properties which has been seen with other species of plants. *In-vitro* anti-oxidant activity of hence, it might help in preventing diabetic complications and serve as a good adjuvant in the present armamentarium of ant diabetic drugs.

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Conflict of Interest

The authors stated that no conflicts of interest.

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