

## SUMMARY AND CONCLUSION

The present work aims at the isolation, structure elucidation of compounds, secondary metabolite profiling, biological and pharmacological activity screening of the extracts and fractionates of *E. crassipes*. *E. crassipes* is an aquatic weed commonly known for its rapid growth and proliferation disrupting the aquatic ecology thereby the economy. In spite of the enormous amount of money spent on controlling the *E. crassipes*, the plant still thrives up disrupting the society. Therefore, better management of the plant by the way of utilizing it, is commendable. From a prospective point, this plant possess several unique properties which makes it a worthy source in many fields like phytoremediation, alcohol production, biogas production, compost, etc.

An extensive review of literature indicated that the phytochemical investigation with the extracts of *E. crassipes* revealed no comprehensive and bulk phytochemical investigation of this plant. Hence, this study was taken up involving the extensive and bulk column chromatography to isolate and characterize the compounds from acetone, ethyl acetate and aqueous extracts and petroleum ether fractionate of ethyl acetate extract of *E. crassipes* and to screen the extracts and fractionates of *E. crassipes* for the biological and pharmacological activities.

The plant was collected in bulk (1050 kg) from a local water body, rinsed thoroughly to remove the debris and roots were cut off. The shade dried (20 days) plant was subjected to alkaline ethanolic extraction which was further extracted with acetone. The bulk extraction was carried out by percolation of the plant (28 kg) with ethyl acetate (160 L) and then with water (160 L) yielding 300 g ethyl acetate extract and 1.20 kg aqueous extract.

### **The summarized results of the present study are given below:**

- ❖ Identifying a suitable method for extraction of *E. crassipes* is crucial as the plant contains about 90 % moisture. The extraction method that best accords extraction of fresh and dried *E. crassipes* was identified. Sonic assisted extraction by ultrasonic homogeniser showed superior extraction efficiency for fresh plant whereas dried plant gave a higher yield in reflux method.

- ❖ Secondary metabolites viz. sterols, alkaloids and terpenoids were isolated from the plant and profiled using GC-MS. The profiling of these metabolites showed the quantitative presence of sterols, alkaloids and terpenoids in the plant.
- ❖ Phytochemical screening of the extracts revealed the presence of metabolites like alkaloids, flavonoids, phenolics, terpenoids, anthraquinones, anthocyanins, proteins, phenolics, quinones and carbohydrates in various extracts of *E. crassipes*.
- ❖ Acetone extract, ethyl acetate extract and aqueous extract were subjected to open column chromatography over silica. The ethyl acetate extract was column chromatographed over activated charcoal and the petroleum ether fractionate was subjected to column chromatography over silica. The isolated compounds were characterized based on its physical characteristics, chemical characteristics and spectral analysis including UV, IR, 1D NMR, 2D NMR and Mass spectra.
- ❖ The open column chromatographic separation of the acetone extract (100 g) afforded a total of 14 fractions of which four were purified and characterized. The compounds were identified as
  - ☞ **Diterpene (A5) - 9 g**
  - ☞ **Undecanoic acid 4-oxo-tetradecyl ester (A15) - 2 g**
  - ☞ **Phthalic acid dibutyl ester (A20) - 8 g**
  - ☞ **Phthalic acid bis-(2-methyl-tridecyl) ester (A22) - 4 g**
- ❖ Column chromatography of the ethyl acetate extract (300 g) over silica resulted in the isolation of 40 fractions of which 8 fractions were purified and characterized. The compounds were characterized as
  - ☞ **A Long chain fatty acid ester (E6) - 440 mg**
  - ☞ **Methyl linoleate (E8) - 2 g**
  - ☞ **Hexadecanyl 2-hydroxy 4-methoxy cinnamate (E10) - 180 mg**
  - ☞ **Mixture of campesterol,  $\beta$ -sitosterol and stigmasterol (E11) – 450 mg**
  - ☞ **Mixture of  $\beta$ -sitosterol and stigmasterol (E12) - 1.5 g**
  - ☞ **Stigmasterol (E13) - 1.5 g**
  - ☞ **Stigmast-22-ene-3,7-dione (E16) - 40 mg**
  - ☞ **An azacrown compound (E21) - 2 mL**

- ❖ Open column chromatography of the aqueous extract (175 g) led to the isolation of one compound (**carbohydrate**) (W5) in high yield (**6.5 g**).
- ❖ The ethyl acetate extract (3.6 g) column chromatographed over activated charcoal yielded 9 fractions and the two fractions were deduced as
  - ⊘ **Tridecanoic acid 3-hydroxy propyl ester (EAC1) - 100 mg**
  - ⊘ **Pentacosan-13-one (EAC2) - 30 mg**
- ❖ The petroleum ether fractionate of ethyl acetate extract (20 g) column chromatographed over silica yielded three compounds:
  - ⊘ **Hydrocarbon (HP1) - 2 g**
  - ⊘ **Heptacosan-14-one (HP6) - 240 mg**
  - ⊘ **Stigmasterol (HP7) - 2 g**
- ❖ HPLC analysis of the ethyl acetate and aqueous extract of *E. crassipes* in the solvent system [TFA / water / acetonitrile] revealed the presence of glutathione in the extracts.
- ❖ The petroleum ether, ethyl acetate, chloroform, ethanol, methanol, aqueous and acetone extract, methanol and ethanol fractionate of aqueous extract of *E. crassipes* and E12 were analysed for their antibacterial and antifungal activity against *Pseudomonas aeruginosa*, *Staphylococcus albus* and *Aspergillus niger*, *Mucor sp* respectively. Acetone extract demonstrated highest activity amongst the extracts whilst chloroform extract, aqueous extract, methanol and ethanol fractionate of aqueous extract showed no activity against the microorganisms tested.
- ❖ The antioxidant activity of the extracts was determined by DPPH radical scavenging assay and reducing power assay. Hydrolysed extract (5.4 µg/mL) displayed highest DPPH radical scavenging ability followed by acetone extract (9.48 µg/mL) compared to the standard (14.43 µg/mL). Acetone extract evinced highest reducing capacity compared to all extracts and standard L-ascorbic acid. The reducing power of the extracts increased with increase in concentration of the extract and time of the reaction.

- ❖ *In vivo* acute toxicity study of the ethyl acetate extract, aqueous extract and methanol fractionate of aqueous extract on Swiss Albino mice revealed the non-toxic nature of these extracts upto a dose of 2000 mg/kg body weight.
- ❖ Four ointments were prepared with paraffin, cetostearyl alcohol and wool fat as vehicle for the extracts of *E. crassipes*. A polyherbal ointment of these extracts with aloe vera, sandalwood and turmeric was prepared for testing its wound healing activity in comparison with ointment containing only *E. crassipes* extracts.
- ❖ Incision wound healing studies on Albino Wistar rats by the topical application of the four ointments indicated the faster wound healing potential of the ethyl acetate extract treated. The tensile strength was high for the rats treated with the ointment prepared with the aqueous extract.
- ❖ Two skin creams were prepared, one with the ethyl acetate extract of *E. crassipes* and the other with ethyl acetate extract, lemon and musk. The skin creams evaluated for their physico chemical parameters conformed to the standard procedure IS 6608:2004. The skin whitening effect of the skin creams by tyrosinase inhibition assay and antiageing effect by DNA damage inhibition and DPPH radical scavenging revealed the potency of these skin creams.
- ❖ The larvicidal, pupicidal and repellent bioassay of petroleum ether extract, acetone extract, ethyl acetate extract, aqueous extract, methanol and ethanol fractionate of aqueous extract of *E. crassipes* against *Culex quinquefasciatus* revealed that ethanol fractionate of aqueous extract of *E. crassipes* was highly effective against all four instar larvae and pupae.
- ❖ *In silico* molecular docking studies of the stigmasterol derivatives against anti-inflammatory enzyme (COX-1, COX-2) and antiageing protein (SIRT1) demonstrated the efficiency of these compounds as potential anti-inflammatory and antiageing agents.
- ❖ All these findings highlight the possibilities to valorize *E. crassipes* for the isolation of compounds in large scale, as it is a source of pharmacologically and industrially indispensable phytochemicals. Fatty esters and sterols were found to be the major

constituent of the plant. **All these compounds were isolated for the first time from *E. crassipes*.** The presence of such compounds of pharmacological importance might have contributed to the biological and pharmacological potential of the extracts.

***The present investigation thus suggests the exploitation of *E. crassipes* as a source of potent phytochemicals through a considerate compromise between the deleterious and meritorious effect of this plant on the environment.***