

Evaluation of Phytochemicals and Brine Shrimp Activity From *Elaphoglossum Beddomei* Sledge

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Summary

The Summary of the present study are subjected to identification of active constituents of secondary metabolites from different polarity of solvent extract of *Elaphoglossum beddomei* leaves were observed. The results of *E. beddomei* leaves were shows on the presence of saponins, tannins, polyuronides, alkaloids, sugars, sterol and triterpenes. Both extract of acetone and alcohol were highly significant active against the cytotoxic activities with the LC₅₀ values of 8.45, and 14.4µg/ml respectively.

Keywords: Phytochemicals; *Elaphoglossum beddomei*; leaves; extracts; brine shrimp activity

Introduction

Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have been served humans as well as valuable source of phytochemicals of secondary metabolites. Even though the availability of different approaches for the drug discovery of therapeutics, plant products still remain as one of the best reservoirs of new structural types. About 25% of all prescriptions sold in the United States are from natural products (1). Currently used for the effective plant drugs such as quinine (2), paclitaxel (Taxol™) (3), camptothecin (4), etoposide (5), mevastatin (6), and artemisinin (7).

Meyer et al. who has been first time *In vivo* lethality test successively studied (8). Recently, cytotoxic and antitumor agents development of several plants such as trilobacin from the bark of *Asimina triloba* (9), *cis*-annonacin from *Annona muricata* (10) and ent-kaur-16-en-19-oic acid from *Elaeoselinum foetidum* (11).

Elaphoglossum beddomei Sledge, which is belong to the family (Lomariopsidaceae), grows in Kakkachi stream, Monjolai Estate, Southern Western Ghats, Tamil Nadu, South India. In the present study, the cytotoxic properties of different polarity of solvents extracts of *Elaphoglossum beddomei* leaves were investigated in phytochemicals analysis and brine shrimp lethality bioassay.

Materials and Methods

Extraction

The collected *Elaphoglossum beddomei* Sledge were air-dried and powdered. 100gms of powdered materials of *E. beddomei* was extracted with different polarity of solvents in Soxhlet apparatus for 8hr. The collected extracts were concentrated in the evaporator under reduced pressure. The qualitative identification of the flavonoids were detected by the method of Shinoda test (1ml of each extract was evaporated, diluted with 1ml of water + 4mg magnesium + 4 drops of concentrated hydrochloric acid). The result of colouring of the solutions from pink to red was an indication for presence of flavonoids. Cyanogenic glycosides were identified by subjecting 1ml of extract in 10ml sterile water and filtered. Sodium picrate was added to the filtrate and heated to boil. The extracts were also tested for carbohydrates using resorcinol solution. Fehling's solution was added to the extract and heated to detect in reducing sugar. Liebermann-Burchardt test was conducted for steroids and terpenoids - to 1ml of each extract of drug, 1ml of chloroform, 3ml of acetic anhydride and 2 drops of concentrated H₂SO₄ acid were added. (Dark green colour solution indicated the presence of Steroids and dark pink or red colouration of the solution indicated the presence of terpenoids). For alkaloids - a few drops of each extract were spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Dragendorff's reagent. (Orange coloration of the spot indicated the presence of alkaloids). Braemer's test was used for identification of tannins: 10% alcoholic ferric chloride solution was added to 3 ml of each extract (Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the extract) (12).

Cytotoxic activity

The cytotoxic effect of different polarity of solvents extract of *E. beddomei* leaves were evaluated by LC₅₀ of brine shrimp lethality tested 12hr. Cytotoxic properties of *E. beddomei* were carried out by brine shrimp lethality bioassay technique against brine shrimp nauplii. *Artemia salina* were placed in a small tank containing 3.8% noniodized NaCl solution for two days to hatched on the shrimp and to be matured as nauplii. 10mg of each tested extracts were dissolved in 200µl of DMSO (dimethyl sulfoxide). Then 100µl of solution was taken in test tube each containing 5ml of seawater and 10 shrimp nauplii. Thus, final concentration of the first test tube solution was 250µg/ml. The positive control solution contained 10 living brine shrimp nauplii in 5 ml sea water. As for negative control, 10µl of DMSO was added to each of the premarked glass vials containing 5ml of seawater and 10 shrimp nauplii. After 24 hours of incubation, the vials were inspected using a magnifying glass and the number of survivors were counted. The concentration-mortality data were analyzed statistically by using Probit analysis for the determination of LC₅₀ values and linear regression for the each extract of *E. beddomei* (8,13,14).

Results and Discussion

The maximum amount of extract of *Elaphoglossum beddomei* leaves found to be acetone and very low extract yield in the both solvents benzene and water seen in table-1. Extracted solvents were treated in both light of ordinary and UV given in the

different color observed. Identification of phytochemical chemicals in the different solvents extract of *Elaphoglossum beddomei* leaves showed the presence of saponins, tannins, alkaloids, sugars, sterol and triterpenes seen in table-2. This study showed more similarity in the phytochemical from leaves of *H. hamiltonii*, *H. nilgirica*, *H. phyllantha* and *H. seratta* (15).

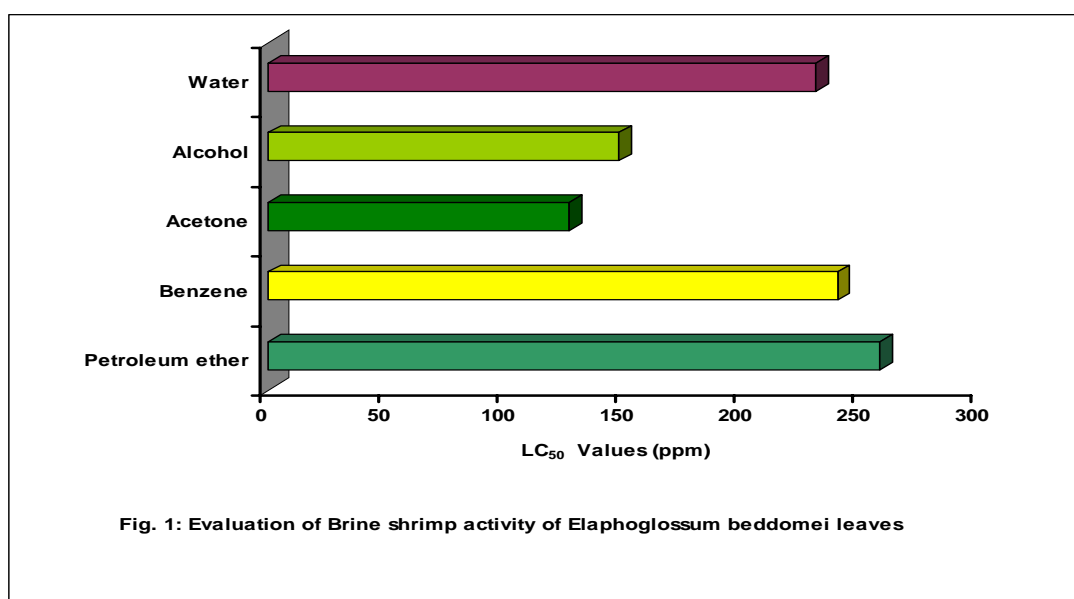
Table 1: Color observation and percentage of different extract from *Elaphoglossum beddomei* leaves

Extract	Yield of the Extract (%)	Color observation of light	
		Ordinary light	UV light
Petroleum ether	2.94	Light green	Light green
Benzene	1.82	Light Yellow	Green
Acetone	5.28	Green	Dark green
Alcohol	4.52	Light green	Yellowish green
Water	1.98	Brown	Yellowish green

Table 2: Identification of phytochemicals from leaves of *Elaphoglossum beddomei*

Compound	PEE	BE	AE	Alcohol	Water
Saponins	-	+	+	-	-
Tannins	-	-	+	-	+
Cyanogenic glycosides	-	-	+	-	-
Alkaloids	+	-	+	+	-
Sugars	+	+	+	+	+
Sterol and triterpenes	-	-	+	+	-

“PEE –Petroleum ether extract; BE- Benzene extract; AE- Acetone Extract”



The present study, both extract of acetone and alcohol were highly significant cytotoxic activities with the LC₅₀ values of 8.45, and 14.4µg/ml respectively. Petroleum ether and benzene extracts showed less significant cytotoxic properties having LC₅₀ values of 34.58 and 28.97µg/ml respectively and moderate activity of water extract of *Elaphoglossum beddomei* seen in Fig.1. Previous report of the plant extracts showed significant lethality against brine shrimp, which has been successfully studied in the biological test to guide the fractionation process of plant extracts to detect the antitumour compound (14). LC₅₀ values <1000ppm are considered significant for crude extracts (8). Our study was agree to previous report on several plant species such as *Baccharis pseudotenuifolia*, *Baccharis ligustrina*, *Baccharis platypoda*, *Baccharis coridifolia*, *Polygala paniculata*, *Polygala sabulosa*, *Croton celtidifolius*, *Cyathea phalerata*, *Trichilia catigua*, *Eugenia uniflora* and *Schinus molle* (16).

Conclusion

The conclusion of the present study are identification of phytochemical identified in different solvent extract of *Elaphoglossum beddomei* leaves showed the presence of saponins, tannins, polyuronides, alkaloids, sugars, sterol and triterpenes. These active constituents may be active against cytotoxic activity. Further studies of the active extracts and isolated compounds in animal models for the therapeutic efficacy and toxicity would provide evidence to determined *E. beddomei* could be beneficial for drug discovery program.

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References

1. Farnsworth NR In Bioactive Compounds from Plants. Ed. Chadwick DJ, Marsh, J, John Wiley, Chichester, 1990: 2-21.
2. Kremsner PG, Winkler S, Brandts C, Neifer S, Bienzle U, Graninger W Clindamycin in combination with chloroquine or quinine is an effective therapy for uncomplicated *Plasmodium falciparum* Malaria in children from Gabon. Journal Infectious Diseases 1994; 169: 467- 470.
3. Wani MC, Taylor HL, Wall ME, Coggon P, McPhail AT Plant Antitumor Agents VI. The Isolation and Structure of Taxol, a Novel Antileukemic and Antitumor agent from *Taxus brevifolia*. Journal of American Chemical Society 1971; 93: 2325-2327.
4. Wall ME, Wani MC, Cook, CE, Palmer KH, Mcphail AT, Sim, GA Plant Antitumor Agents I. The Isolation and structure of camptothecin, a novel alkaloidal leukemia and tumor inhibitor from *Camptotheca acuminata*. Journal of American Chemical Society 1966; 88: 3888-3890.

5. Endo A, Kuroda M, Tsujita YY ML-236A, MI-236B, and ML-236C New Inhibitors of Cholesterologenesis produced by *Penicillium citrinum*. *Journal of Antibiotics* 1976; 29: 1346-1348.
6. Keller-Juslen C, Kuhn M, Von Wartburg A, Stähelin H, Synthesis and antibiotic activity of glycosidic lignan derivatives related to Podophyllotoxin. *Journal of Medical Chemistry* 1971; 14: 936-940.
7. Klayman DL Qinghaosu (Artemisinin): An Antimalarial Drug from China. *Science* 1985; 228: 1049-1055.
8. Meyer BN, Ferrighi NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL Brine shrimp: A convenient general bioassay for active plant constituents. *Planta Medica* 1982; 45, 31-34.
9. Zhao G, Hui Y, Rupprecht JK, McLaughlin JL, Wood KV Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. *Journal of Natural Products* 1992; 55: 347-356.
10. Mongelli E, Pomilio AB, Sanchez JB, Guerra FM, Massanet GM ent-Kaur-16-en-19-oic acid, a KB cells cytotoxic diterpenoid from *Elaeoselinum foetidum*. *Phytotherapy Research* 2002; 16: 387-388.
11. Rieser MJ, Gu ZM, Fang XP, Zeng L, Wood KV, McLaughlin JL. Five novel mono-tetrahydrofuran ring acetogenins from the seeds of *Annona muricata*. *Journal of Natural Products* 1996; 59: 100-108.
12. Maridass M, Zahir Hussainb MI, Raju G Phytochemical Survey of Orchids in the Tirunelveli Hills of South India. *Ethnobotanical Leaflets* 2008; 12: 705-12.
13. Persoone G. et al. Proceeding the international symposium on brine shrimp *Artemia salina*, volumes 1-3, Universe press. Witteren, Belgium, 1980: 1-3.
14. McLaughlin JL Bench tops bioassay for the discovery of bioactive compounds in higher plants. *Brenna* 1990: 29.
15. Maridass M, Raju G Investigation of Phytochemical and Antimicrobial Activity of *Hubertia* Species. *Pharmacologyonline* 2009; 3: 688-692.
16. Pimentel Montanher AB, Pizzolatti MG Costa Brighente IM An Application of the Brine Shrimp Bioassay for General Screening of Brazilian Medicinal Plants. *Acta Farm. Bonaerense* 2002; 21(3): 175-8.