Evaluation of growth performance and saponin composition in *Bacopa monnieiri* (Scrophulariaceae) cultivated under hydroponics

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Abstract

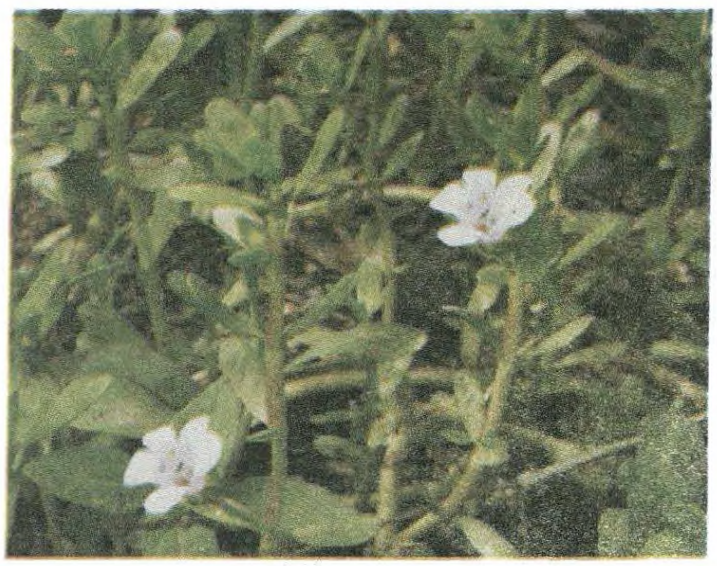
The medicinal plant *Bacopa monnieiri* (Lunuwila) of the family Scrophulariaceae has many biologically active compounds such as anticancer, cognitive enhancing and anti-epilepsy compounds. It is used in Ayurveda system of medicine in Sri Lanka. Although annual demand for *B. monnieiri* is growing exponentially, there is no commercial cultivation recorded in Sri Lanka. Objective of the study is to evaluate the growth, yield and chemical composition of *B. monnieiri* when cultivated under hydroponic conditions. Quantitative analysis was carried out to compare growth and yield measurements such as total fresh and dry weight, shoot: root ratio, leaf: stem ratio, number of leaves, number of shoots, shoot length and moisture percentages of hydroponically grown plants and plants grown under marshy soil. Thin Layer Chromatography analysis was done to qualitatively compare saponins when cultivated under different conditions. Results showed that the growth and yield parameters of *B. monnieiri* were significantly higher *(*$P<0.05$*)* in hydroponically grown plants than that of wild plants. Saponins of *B. monnieiri* have not shown much difference between hydroponically grown plants and wild plants. Therefore, the results revealed that the hydroponics technique is biologically feasible for the cultivation of *B. monnieiri* and the major phytochemical saponin has not changed much when *B. monnieiri* was cultivated under hydroponics.

Introduction

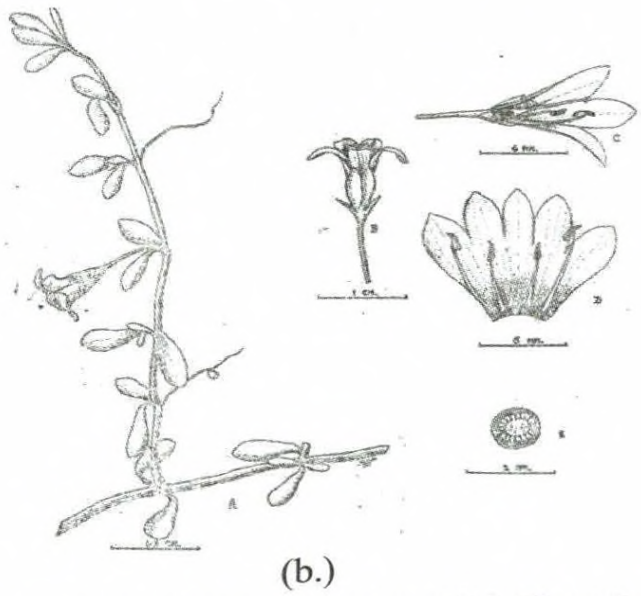
The medicinal plant *Bacopa monnieiri* L. Pennell (Lunuwila) belongs to family Scrophulariaceae is a palaeotropical species distributed in India, Sri Lanka, Malaya and Philippine islands [1]. The species is found in open marshy places, along banks of ponds and creeks of the lowlands close to the coast. It is a gregarious plant because of its creeping habit covers large tract of ground, particularly in marshy localities. It is very common in low-country in Sri Lanka. Plant specimens were previously collected and examined from Mannar, Putttlam, Nuwara eliya, Kaluthara and Galle districts of Sri Lanka [2].

As shown in the Figures 1(a) and 1(b), *B. monnieiri* is an annual aquatic herb consists of a prostrate stem, spatulate or obovate - oblong leaves. Further the leaves are obtusely rounded at the apex and glabrous. Pale violet - blue flowers have sepals divided to base, broadly companulate corolla tube with two - lobed upper lips. Stamens of the flowers are inserted at top of corolla tube. Capsule is oblong - avoid in shape [1]. It Flowers throughout the year [2].

The entire plant is used in Ayurveda and indigenous system of medicine in Sri Lanka and elsewhere [1]. The plant possesses astringent, anticancer, cognitive enhancing, anti-hepatic properties [3, 4, 5]. The phytochemical responsible for anticancer activity called bacoside A (containing bacoside A3) is isolated from the whole plant of *B.*



(a.)



(b.)

Figure 1: *Bacopa monnieiri* Morphology: (a.)Vegetative and reproductive morphology (b.) Plant habit: A, twig with leaves and flower. B, flower lateral view. C, longitudinal section of flower. D, corolla opened out showing stamens. E, transverse section of ovary (source: Jayaweera, 1982)

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monnieiri. Terpenoids (bacosides) are the major constituent of *B. monnieiri* act like taxol, which are currently being used in cancer chemotherapy [3], Triterpenoid saponins and bacosides of *B. monnieiri* play key role for enhancing nerve impulse transmission. Bacosides support the repair of damaged neurons by enhancing kinase activity, neuronal synthesis, restoration and regeneration of synaptic activity resulting in nerve impulse transmission [4]. This plant showed important antioxidant activity in brain parts like hypothalamus striatum and frontal cortex. Further, studies showed its protective effect against DNA damage in astrocytes and fibroblast cells [5]. Triterpenoid saponin is the major secondary metabolites in *B. monnieiri* reported to be responsible for the cognitive enhancing activity of *B. monnieiri*. The saponins designated as bacoside-A and bacoside- B, betulic acid, D-manitol, stigmastarol and beta-sitosterol was isolated from the whole plant [6]. It is suggested to be useful in hepatitis, urinary tract infections, high blood pressure, rheumatism, congestive heart failure and cerebral palsy [7]. Ayurvedic treatment regimen is effective than physiotherapy treatment in the management of cerebral palsy [7].

In Sri Lankan Ayurveda system of medicine *B. monnieiri* is used for treatments and a large number of plants are required in Sri Lankan Ayurvdic hospitals. It has been estimated that annual demand of Ayurvedic drug co-operation for *B. monnieiri* is about 1700 kg [8]. The entire requirement is fulfilled from the natural population and it has lead to a rapid depletion of the plants from its natural habitat. Furthermore, International Union for conversation of Natural Resources has listed *B. monnieiri* as a threatened species [9]. However, there is no commercial cultivation or agronomic practices of *B. monnieiri* recorded in Sri Lanka or elsewhere. This can be one of the reasons of exploitation of *B. monnieiri*. Moreover, there is no information on processing of this plant on its biomass and chemical constituents.

Anyway, herbal ingredients from cultivated plant are sometimes considered qualitatively inferior when compared with naturally grown plants [10]. Therefore, the present study is an attempt to qualitatively and quantitatively assess chemical composition and the growth performance of *B. monnieiri* cultivated in hydroponics with plants grow in natural habitats.

Materials and Methods

Plants were collected from a pond at Bandaranayke Memorial Ayurvedic Research Institute (BMARI), Nawinna, Maharagama. Plants were identified from: herbarium of BMARI based on both vegetative and reproductive characters of *B. monnieiri*.

The study was conducted in a plant house (10m x 12m) at the Botany division of BMARI. *Bacopa monnieiri* were grown under commercially available Albert's solution in Sri Lanka (CIC Fertilizer Pvt. Ltd.). The experiment was conducted in a Complete Randomized Design (CRD). Three replicates of Styrofoam boxes (each replicate box contained six plants) were allocated for each treatment. Seventy two *B. monnieiri* cuttings (10 cm length with 10 leaves) were randomly selected to grow under hydroponically and eighteen cuttings were selected to grow under natural marshy condition as control. The five treatments imposed were as follows.

Tratment - 1 (T1)
100% concentration of Albert's solution
Tratment - 2 (T2)
75% concentration of Albert's solution
Tratment - 3 (T3)
50% concentration of Albert's solution
Tratment - 4 (T4)
25% concentration of Albert's solution
Tratment - 5 (T5) Control
Natural marshy soil medium

100% concentration of Albert's solution (T1) was prepared according to the Department of Agriculture (DOA) recommended composition and other treatments T2, T3 and T4 were prepared by diluting it further with water. The Electrical conductivity and pH values were measured twice a month. The optimum values of Electrical Conductivity and pH are (1.5 - 2.5ds/m), (5.8 - 6.5) respectively [11]. Quantitative and qualitative evaluation of harvested plants was done in the laboratory.

Growth and Yield Measurements

Total fresh and dry weights, shoot: root ratio, leaf: stem ratio, number of leaves, number of shoots, shoot length and moisture content of Hydroponically Grown Plants (HGP) and Soil Grown Plants (SGP) were assessed.

Statistical analysis

Data was tabulated, and analyzed using Analysis of Variance (ANOVA) procedure of Statistical Analysis System (SAS). The Least Significant Difference (LSD) test was used to compare differences among the treatment means at $p=0.05$

Thin Layer Chromatography (TLC) Analysis

Thin Layer Chromatography (TLC) analysis of whole plant was carried out for all treatments to check whether the chemical constituents, saponins of *B. monnieiri* are affected by the hydroponics culture.

Preparation of *B. monnieiri* extraction for TLC analysis

Powdered plant material (2 g) was extracted by heating at 100 °C for 10 minutes under reflux with 10 ml of 70% ethanol. Then the extraction was filtered and the clear filtrate was evaporated up to 5 ml, and 25-40 µl of that solution was applied for chromatography [12].

Preparation of solvent systems

Three solvent systems for chromatography were optimized for TLC. The solvent systems used for analysis were mixtures of chloroform: methanol: water (64: 50: 10, v/v), chloroform: methanol: water (70: 30: 4, v/v) and chloroform: methanol: (95: 5, v/v) [12]. The R_f (retention factor) values were calculated using the following formula.

$$R_f \text{ value} = \frac{\text{Distance between origin and the centre of the spot}}{\text{Distance between origin and solvent front}}$$

TLC plates were observed under UV (λ=365 nm) and spraying reagents such as anisaldehyde, sulfuric acid, glacial acetic acid and methanol under visible light [13].

RESULTS

Growth and Yield Measurements

Results of the quantitative analysis are shown in Table 1 and 2.

Table 1: Assessment of growth parameters of Hydroponically Grown Plants (HGP) and Soil Grown Plats (SGP) of *B. monnieiri*

Tratments	Fresh Weight (g)	Dry Weight (g)	Shoot : Root ratio	Leaf : Stem ratio
(a). HGP				
T1 - (100%)	49.86^{b}	10.49^{b}	2.54^{bc}	0.67^{a}
T2 - (75%)	47.94^{b}	9.86^{b}	2.71^{b}	0.57^{a}
T3 - (50%)	71.41^{a}	14.24^{a}	3.85^{a}	0.56^{a}
T4 - (25%)	47.03^{b}	10.67^{b}	2.90^{b}	0.61^{a}
(b) SGP				
T5 - (Control)	12.77^{c}	3.86^{c}	1.91^{c}	0.66^{a}
LSD	16.708	2.321	0.650	0.183
CV	20.050	12.989	12.793	16.377

Note: Within each column, means followed by the same letter are not significantly different at p=0.05 and the different letters show significantly different values. Measurements are the means of three replicates (each replicate comprised of six plants). CV= coefficient of variance

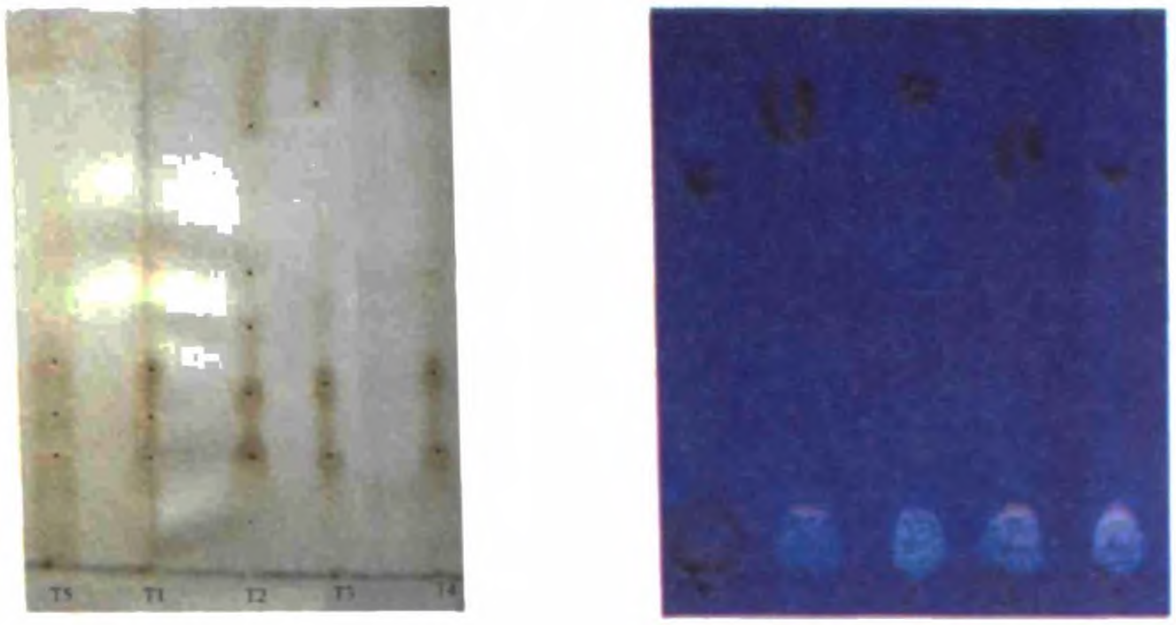
Table 2: Number of leaves, number of shoots, shoot length and moisture percentage of Hydroponically Grown Plants (HGP) and Soil Grown Plants (SGP) of *B. monnieiri*

Tratments	Number of leaves	Number of shoots	Shoot length (cm)	Moisture content (%)
(a). HGPs				
T1	318.00^{b}	39.11^{b}	139.49^{b}	78.43^{a}
T2	332.39^{ab}	41.22^{b}	140.33^{b}	78.81^{a}
T3	374.33^{a}	4189^{a}	163.33^{a}	80.11^{a}
T4	314.67^{b}	36.61^{b}	033.44^{b}	76.51^{ab}
(b) SGPs				
Control (T5)	142.44^{c}	25.77^{b}	68.16^{c}	69.65^{b}
LSD	49.955	9.733	10.249	7.274
CV	9.265	14.490	4.369	5.123

Note: Within each column, means followed by the same letter are not significantly different at p=0.05 and the different letters show significantly different values. Measurements are the means of three replicates (each replicate comprised of six plants).

TLC Analysis

Thin Layer Chromatograms of the extracts at UV (λ= 365nm) showed several spots as in the Figures 2(a), 3(a) and 4(a). The R_f vales of the each extract is given in the Tables 3, 4 and 5. The same TLC plates observed under visible light after spraying of anisaldehyde [13] are shown in Figures 2(b), 3(b) and 4(b).



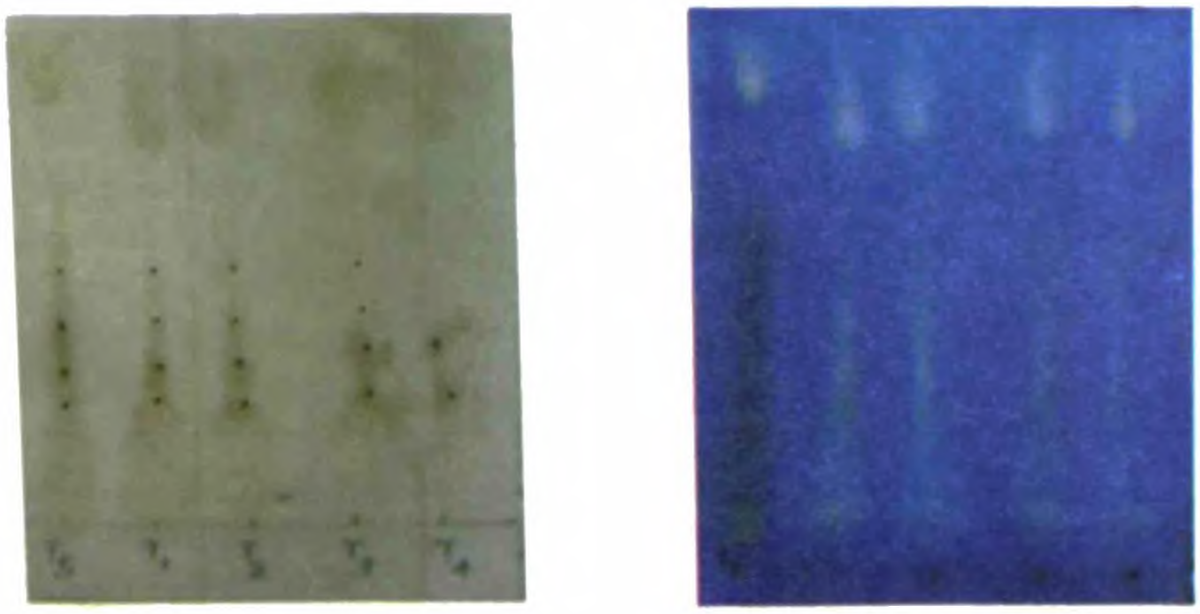
(a) (b)

Figure 2: TLC using solvent sample I - chloroform: methanol: water (64: 50: 10, v/v)(a.) Thin layer chromatogram of five ethanol extracts of *B. monnieiri* developed in solvent system I and visualized under visible light after spraying anisaldehyde reagent. (b.) Thin layer chromatogram of five ethanol extracts of *B. monnieiri* developed in solvent system I and detected under UV (λ=365 nm).

Table 3: R_f values of *Bacopa monnieiri* extracts developed under solvent system I

Exteact sample	R_f values						
T1	-	0.16	0.23	0.36	0.58	0.58	-
T2	-	0.16	0.23	0.36	0.58	0.58	0.89
T3	0.05	0.16	0.23	0.36	0.58	0.58	0.89
T4	-	0.16	0.23	0.36	0.58	0.58	0.89
T5	0.05	0.16	0.23	0.36	0.58	0.58	-

The results of TLC of T1 to T4 extract and T5 developed under solvent system II (Figure 3) and spraying with anisaldehyde reagent could be observed purple spots. TLC pattern of T1, T2 and T3 were the same and were also similar to TLC pattern of T5.



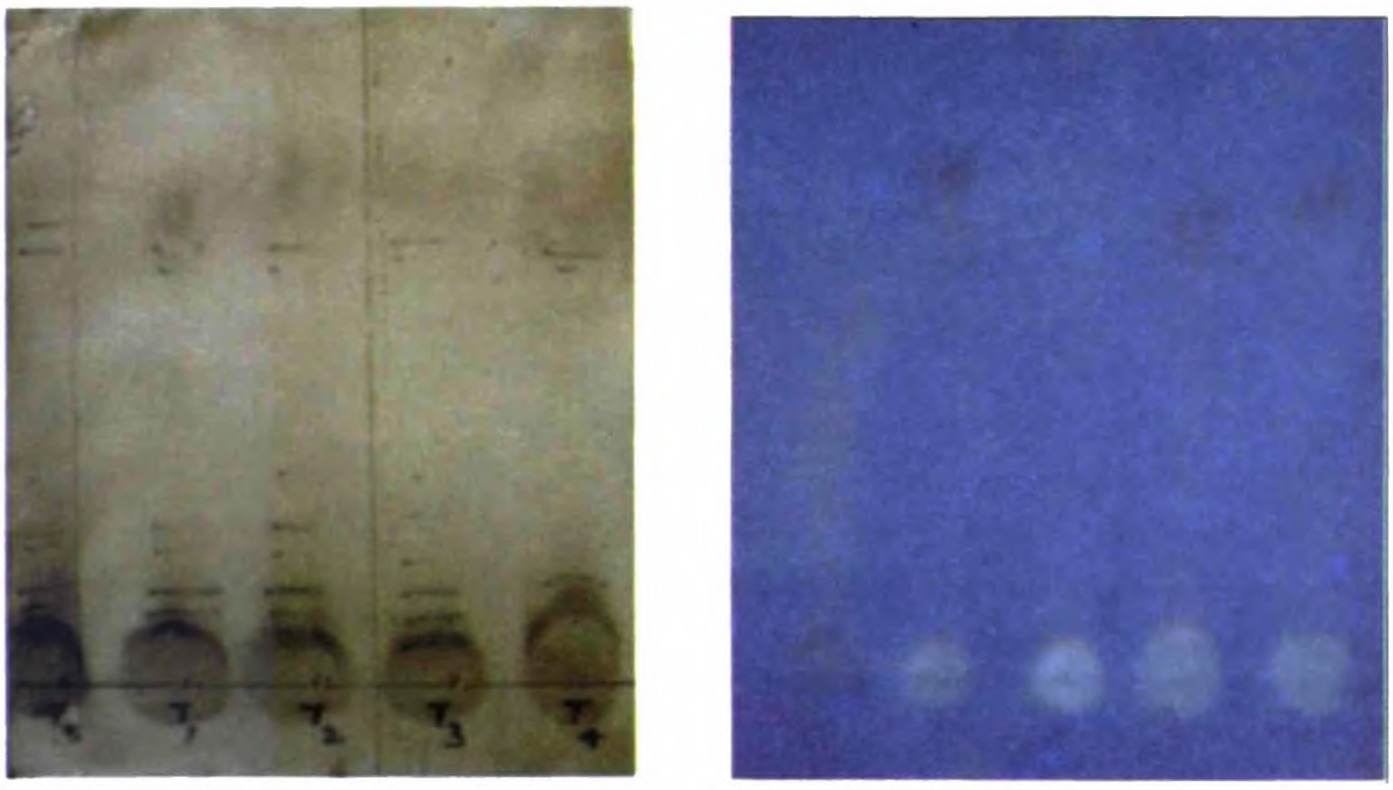
(a) (b)

Figure 3: TLC using solvent sample II- chloroform: methanol: water (70: 30: 4, v/v)

Thin Layer Chromatogram of five ethanol extracts of *B. monnieiri* developed in solvent system II and detected under visible light after spraying anisadehyde reagent. (b.) Thin Layer Chromatogram of five ethanol extracts of *B. monnieiri* developed in solvent system II and detected under UV (λ=365 nm).

Table 4: R_f values of *Bacopa monnieiri* extracts developed under solvent system II

Exteact sample	R_f values				
T1	0.19	0.25	0.32	0.42	0.53
T2	0.19	0.25	0.32	0.42	0.53
T3	0.19	0.25	0.32	0.42	0.53
T4	0.19	0.25	-	-	-
T5	0.19	0.25	0.32	0.42	0.53



(a) (b)

Figure 4: TLC using solvent sample III- chloroform: methanol (95: 5, v/v)
Thin layer chromatogram of five ethanol extracts of *B. monnieiri* developed in solvent system III and detected under visible light after spraying anisaldehyde reagent. (b.) Thin layer chromatogram of five ethanol extracts of *B. monnieiri* developed in solvent system III and detected under UV (λ=365 nm).

Table 5: R_f values of *Bacopa monnieiri* extracts developed under solvent system III

Exteact sample	R_f values						
T1	0.07	0.10	0.14	0.21	0.31	0.63	0.68
T2	0.07	0.10	0.14	0.21	0.31	0.63	0.68
T3	0.07	0.10	0.14	0.21	0.31	0.63	0.68
T4	0.07	0.10	0.14	-	-	0.63	0.68
T5 (control)	0.07	0.10	0.14	0.21	0.31	0.63	0.68

Discussion

Growth and Yield Measurements

According to the Table 1, hydroponically grown plants (T1 –T4) showed a higher dry matter accumulation than that of the soil grown plants. Many crops showed at least more than two times higher yield under hydroponics than under soil culture [14]. Among the different treatments tested, plants grown in 50% concentration of Albert's solution showed the highest dry matter accumulation while the lowest was shown in soil grown plants/control (T5). Furthermore, shoot: root ratio in HGP was higher than that of SGP (control). As the shoot: root ratio represents the proportion of total biomass allocated to shoots and higher values observed in HGP. The leaf: stem ratio values of HGP and SGP were not significantly different ($p>0.05$). According to the Table 2, number of leaves of hydroponically grown plants (T1-T4) showed significantly higher value than that of soil grown plants (T5). Number of leaves indicates the strength of the source of a crop due to its ability of provides the platform for photosynthesis. In addition photosynthesis of a plant is proportional to the amount of leaves on the plant [15]. Among the hydroponically grown plants, (T3) was shown the highest and (T5) was shown the lowest value of number of leaves. Number of shoots and shoot length were shown same results. The lowest and highest values were shown (T3) and (T5) respectively. According to the results, moisture percentage of hydroponically grown plants showed higher value than that of soil grown plants. Moisture content of hydroponically grown plants vary between 76.51% and 80.11% and soil grown plants showed the lowest value (69.64%). When using dry form of *B. monnieiri* plants for medicinal purpose, high moisture content can be disadvantageous, but when considering the yield of hydroponically grown plants, that was higher than that of soil cultivation. However, dry matter accumulation/ dry weight value of hydroponically grown plants also in higher level than that of soil grown plants. Therefore a high moisture percentage is not much disadvantageous.

TLC Analysis

Saponin is the major compound that could be seen in *B. monnieiri* extracts as purple colored spots under visible light [13]. According to the Table 3, spraying the chromatogram with each reagent revealed purple spots with R_f values ranging from 0.16 to 0.90. Purple spots were observed with R_f values 0.05, 0.16, 0.23, 0.36, 0.58 and 0.90. These bands are for different kinds of saponins in *B. monnieiri*. In chromatograms of plants grown under the treatment T1, the first band belonged to R_f value 0.05 was absent and the other bands were similar to the TLC pattern of SGP. In the chromatogram of T2, the lowest R_f value was 0.16 and highest was 0.89. On the other hand chromatogram of the treatments T1 and T4 were the same.

According to the Table 4, T1, T2 and T3 were the same and were also similar to TLC pattern obtained with T5. Purple spots with R_f values of 0.19 to 0.53 and the other spots of chromatograms with R_f values 0.25, 0.32 and 0.42 were observed. However, the TLC pattern of T4 was different than that of the others. Because, in the chromatogram of T4, R_f values 0.05 and 0.90 were absent.

According to the Table 5, R_f values had 0.07 to 0.63 range. The results also showed that T3 was somewhat similar to the T5. The R_f values of T1, T2, T3 and T5 were also the same. However, there were bands responsible for the R_f values 0.07, 0.10, 0.14 and 0.63 only in the chromatogram of T4 and bands belonging to the R_f values of 0.21 and 0.31 were absent in T4.

Conclusion

Growth and yield parameters of *B. monnieiri* were significantly higher ($p<0.05$) in the hydroponically grown plants than that of plants grown under natural marshy soil medium. Results of TLC analysis for saponin did not show a significant difference between hydroponically and soil grown plants. Therefore, according to the experimental evaluation in this study, *B. monnieiri* cultivated under hydroponics have shown high growth and yield performance than plants grown in marshy lands and saponin content was not significantly different in both conditions.

Acknowledgement

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References

1. Jayaweera DMA. Medicinal plants (Indigenous and Exotic) used in Ceylon, The National Science Council of Sri Lanka, Colombo, 1982; Part IV. 72- 73.
2. Cramer LH. Scrophulariaceae. In: Dassanayake MD, Fosberg FR, editors. A revised hand book to the Flora of Ceylon, Oxford and IBH Publishing Co., New Delhi, India, 1981; 4: 421-422.

3. Prakash NS, Sudaram R, Mitra SK. *In vitro* and *In vivo* anticancer activity of bacoside A from whole plant of *Bacopa monnieiri* (Linn), *American Journal of Pharmacology and Toxicology*. 2011: 6.

4. Roodneys S, Booth D and Bulzomi S. Chronic effects of *Bacopa monnieiri* on human memory, Woolangong, Australia,2002 [Internet] available from: http://www.holistic-herbalist.com/bacopahtml. Accessed date: 01 Dec. 2011.

5. Nathan PJ, Tanner S and Lloid J. Effect of a combined extract of Ginko biloba and *Bacopa monnieri* on cognitive fuction in healthy humans, *Human psychophamacology: Clinical and Experimental*, Victoria, Australia. 2004; 19: 91-96.

6. Kapoor LD. Ayurvedic Medicinal Plants, CRC press, Washington D.C, USA. 1990; 61.

7. Weerakoon S and Amarasinghe APG. Study of the efficacy of an ayurvedic treatment regimen on Balaka Pakshagatha with special reference to cerebral palsy. *Sri Lanka Journal of Indigenous Medicine* 2011; 1(2): 55-58.

8. Anonymous Compendium of Medicinal Plants, A Sri Lankan Study. Ayurvedic Drug Co-operation, Sri Lanka. 2004; 4: 39-41.

9. Joshi AG. Ashutosh RP. Asha M. Environmental and experimental biology, High Frequency of Shoot Regeneration on Leaf Explants of *Bacopa monnieri,* Baroda, India. 2010: 81-84.

10. Schippmann U, Leaman DJ and Cunningham AB. Impact of cultivation and gathering of medicinal plants on biodiversity: Global trends and issues, Inter Departmental Working Group on Biological Diversity for Food and Agriculture, Rome. 2002.

11. Anonymous. Hydroponics. Extension and Training Centre, Department of Agriculture, Sri Lanka. 2002.

12. Wanger H, Bladt S and Zgaiki EM. Plant drug analysis: A Thin Layer Chromatography Atlas, Springer, Berlin, Germany 1984: 226-299

13. Resh HM. Hydroponics food production. A definitive guide book of soil-less food growing method.6th ed. Woodbridge Press Publishing Company. California 2001.

14. Padmathilake KRE, Wickramarachchi VN and Bandara DC. Biological and economical feasibiltity of growing mint (*Mentha sylvestris* L.), mustard (*Brassica intergrifolia* L.) and asamodagam (*Trachyspermum involucratum* L.) under hydroponics, *Tropical Agriculture Research* 2007; 19: 193-201.

15. Anees AS. Pharmaceutical Analysis. 2nd ed. Department of Pharmaceutical Chemistry, New Delhi, India 2010: 198-213.