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RESEARCH ARTICLE

Impact of chemical stress on secondary metabolites of *BACOPA MONNIERI* (L.) WETTST

Anupama, V.C., Sreenand, T.M. and Revathi, P.*

Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore-641 029, Tamil Nadu, India

ABSTRACT

The demand for cultivating medicinally important plants has a lot of scope and significance at present and in the near future. The present study has been carried out to check the impact of chemical stress on secondary metabolites in medicinally important plant *Bacopa monnieri* (L.) Wettst. As the initial step the plant was cultivated in optimum environment conditions. They were subjected to stress by applying chemicals such as urea and salt in separate pots in different doses. The urea and salt stress has induced several biochemical changes that lead to morphological changes. The morphological observations showed presence of yellow colour in leaves. But there were no any serious effects observed in plant life. After stress application plants were collected separately and it was shade dried, powdered and stored separately. Using the powdered sample and ethanol extract of the plant the total phenolic contents, flavonoids, terpenoids, alkaloids and saponins were evaluated and compared the control and plants subjected to stress. Higher the stress gives higher content of secondary metabolites except the few. The study proves that there is production of secondary metabolites during stress conditions in the plant *Bacopa monnieri*. The production is higher in such treated plants when compared to plants kept as control. This piece of research has paved a way for its improved commercial production. Further the researchers can also proceed with other research analysis based on compounds synthesized through application of different stresses to this plant.

Keywords: *Bacopa monnieri,* Secondary metabolites, Flavonoid, Alkaloid, Terpenoid, Saponin**,** Urea Stress, Sodium Chloride Stress

1. INTRODUCTION

In the present world several researches and experiments are going on that are mainly focusing on how the life of people can be improved and make them free of diseases and other health related problems. In order to make life healthier people prefer traditional medicines made from medicinal plants other than pharmaceutically synthesized chemicals. Therefore, medicinal plants have always played a pivotal role as sources of drug lead compounds. Early humans, driven by their instinct, taste and experience, treated their illness by using plants; hence the history of medicinal plants is as long as the history of humans [1]. Demand for the medicinal plant is increasing with expansion in human needs, numbers and trade purposes. So many plants, especially wild species are more prone to harvesting and further synthesis of medicines. Therefore, cultivation of medicinal plants can decrease the amount to which wild populations are harvested. It will also help to preserve plant species from extinction and will promote socio economic growth [2]. Cultivating plants on a large scale would provide enough raw materials for the pharmaceutical industries. Systemic cultivation ensures the quality and purity of medicinal plants. Thereby collection of raw materials of crude drugs from cultivated plants gives a better yield in therapeutic quality.

Secondary metabolites are a diverse group of chemicals like phenolic compounds, alkaloids, flavonoids, saponins, etc. synthesized by plants mainly as defence compounds. The medicinal plants are rich in secondary metabolites. Alkaloids are secondary metabolites biosynthetically derived from amino acids resulting in a variety of chemical structures [3]. They have basic properties in which they are water soluble under neutral acid conditions and lipid soluble under neutral and basic conditions. This is especially important for dissolution in protonated form and membrane permeation in deprotonated form [4]. Abiotic stress caused by

*Correspondence: Revathi, P., Department of Botany, Kongunadu Arts and Science College (Autonomous), Coimbatore - 641029, Tamil Nadu, India. E.mail: revathip_bo@kongunaducollege.ac.in

deficiencies or excesses in environmental factors including water, salt, light, temperature, and nutrients can substantially reduce plant growth and productivity and even survival [5]. Any abiotic stress or any change in the normal biological factor in the environment alters the different physiological, biochemical and metabolic function of plants and affect the plant growth where, plants protect themselves by acquiring various defence mechanism to prevent their negative effect on growth and production [6].

Salinization of soil or water adversely affects the photosynthesis, respiration, morphological and biochemical characteristics of the plants [7]. Salt stress often creates both ionic as well as osmotic stress resulting in accumulation or decrease of specific secondary metabolites in plants [8]. Alkaloids are found in about 20% of plant species in small quantities [9] and their production, extraction and processing remain major areas of research and development [10]. Saponins are another class of complex and chemically diverse group of compounds. The demand for saponins is increasing in medicinal research and applications. They are considered as the starting precursor for the semi-synthesis of steroidal drugs in the pharmaceutical industry [11]. Secondary metabolites play a major role in the adaptation of plants to the environment and in overcoming stress conditions [12]. In the growth conditions of plants, numerous secondary metabolites are produced by them to serve a variety of cellular functions essential for physiological processes. The type and concentration of secondary metabolites produced by a plant are determined by the species, genotype, physiology, developmental stage and environmental factors during growth. Recent researchers have found that stress and the subsequent defense response have led to the production of secondary metabolites in plants [13]. This implies that plants subjected to stresses can produce such compounds. Salt stress often creates both ionic as well as osmotic stress in plants. It leads to cellular degradation and reduction of cytosolic and vacuolar volumes resulting in accumulation or decrease of specific secondary metabolites in plants [8].

Application of nutrient elements can also affect the production of secondary metabolites. Incorporation of one or more trace elements could increase or decrease the production of secondary metabolites depending on the plant species as well as concentrations of these elements [14]. Plants produce increased secondary metabolites when exposed to stressful growth condition. For example, the salinity stress has induced the production of saponins, flavonoids, phenolics and photosynthetic pigments [15], the nitrogen fertilizer in excess amount has induced production of alkaloids [16]. Hence this stress application is being one of the main inductive techniques to enhance the secondary metabolites in many medicinal plants. This technique will aid to increase the natural compound concentration and compromise the demands in pharmaceutical industries and assist in farmers' welfare.

As a medicinally important plant which yield several secondary metabolites that are commercially important *Bacopa monnieri* (L.) Wettst was choosen to estimate the quantity of alkaloids and saponins on chemical stress application. Water hyssop and "Brahmi" are the two words used for *Bacopa monnieri* in the tradional system of medicine. According to National Medicinal Plants Board, New Delhi *Bacopa monnieri* (L.) Wettst. is identified for cultivation and conservation and denoted among the list of medicinal plants in high volume trade i.e., 2000-5000 metric ton annually. The whole plant including flowers can be used for medicinal purposes. It is used as a nerve tonic and cure for epilepsy, insanity, anaemia, dermatosis, arthritis, rheumatism, asthma, cough, diabetes, snakebite and ulcers. The plant contains alkaloids like brahmine, herpestine, nicotine and saponins like bacoside A and B, hersaponin, triterpenoid saponin and other compounds like stigmasterol, monnierin and α-mannitol. The plant is used as the principal ingredient of classical Ayurvedic preparations like Brahmighritam, Brahmirasayanam, Brahmi taila, Brahmi sarbat, etc. 'Memory Plus' is a product marketed in India that contains the standardised extracts of bacosides obtained from this plant which was made under the guidelines of herbal medicines issued by WHO. Because of its inherent potential in enhancing memory and vitality this plant has gained attention for its commercial cultivation globally. Stress is considered as a negative factor being responsible for severe yield losses in agriculture. However, with respect to spice and medicinal plants stress induces the production of secondary metabolites desirably. Based on these novel insights, stimuli for practical approaches for enhancing the production of secondary metabolites focused by deliberately applying moderate stress during their cultivation, *Bacopa monnieri.*

2. MATERIALS AND METHODS

2.1. Plant Description

The plant *Bacopa monnieri* is a small succulent herb with annual creeping nature. *Bacopa monnieri* also referred to as water hyssop and "Brahmi," has been used in the Indian system of medicine since time immemorial. It belongs to the family Scrophulariaceae and is found in wet, damp, and marshy areas. *Bacopa monnieri* is conventionally used for diverse ailments, but is best known as memory enhancer. A vast range of studies using methanolic and ethanolic extracts of this plant have shown its effect in treatment of wide range of diseases like diabetes, depression, cancer, inflammation etc. [17].

2.2. Collection of Plant Species

The plant *Bacopa monnieri* (L.) Wettst. was collected from Thiruvambady, Kozhikode (Dist), Kerala and grown under optimum growth conditions. The plant prefers wet and semi shade conditions with a temperature range between 15-40°C, humidity 65-80% and clayey loamy soil with acidic nature.

2.3. Cultivation Practices

Soil and climate, land preparation, Irrigation, weed control, fertilizer requirement, harvesting method are followed in standardized methods prescribed by National Medicinal Plants Board, New Delhi and Central Institute of Medicinal and Aromatic Plants, Resource Centre, Bangalore. Before removing the cuttings from the nursery, it should be flooded. The plants are dug out taking care to minimize the damage to the roots of the cuttings. Plant cuttings about 6-8cm long, containing nodes with roots are used for transplanting. One day before planting, vermicomposting is spread on the surface of the plots and mixed thoroughly with top 10 cm soil and then the land is flooded. The cuttings are transplanted in wet soil at spacing of 15 ×15 cm. Flood irrigation should be provided immediately after planting. For its proper growth daily irrigation, organic manuring and hand weeding should be done. By preferring all these conditions 10-15 stems of equal length were grown in fifteen different pots numbered 1 to 15 each filled with 5kg of soil on 09/12/2021.

2.4. Stress Application

The cultivated plantlets started to grow after three weeks (30/01/2022). Then the plants were subjected to chemical stress using urea and NaCl. The pots numbered 1, 2, 3 were kept as control. The normal level of urea preferred by the plant is 50mg urea/kg of soil. So the pots numbered 4, 5, 6 were treated with 100mg urea/kg of soil and the pots numbered 7, 8, 9 were treated with 200mg urea/kg of soil. The normal level of salt concentration that the plant prefers is below 100mM NaCl/L. So the pots numbered 10, 11, 12 were treated with 300mM NaCl and the pots numbered 13, 14, 15 were treated with 400mM NaCl. The growth of the plant was observed for another five days. Presence of yellow coloured leaves was observed in some stems of plants treated with urea and NaCl.

2.5. Preparation of Sample

After five days of observation, (on 4/2/2022) the plants kept as control and those subjected to chemical treatment were collected separately. Later kept for shade dry by storing separately. The dried samples were then powdered using a motor and pestle. Each sample was then weighed and stored separately. A part of sample has taken for soxhlet extraction using ethanol solvent, the extract has stored for certain quantification studies.

2.6. Quantitative Estimation of Phenolics

The sample extracts (50 µl of different solvent extracts) were added with Folin Ciocalteu reagent (0.5 ml, 1:1 diluted with distilled water) for 5 min and aqueous Na_2CO_3 (2.5 ml, 20%) was added. The mixture was vortexed, allowed to stand for 40 min at room temperature in dark and the phenols were determined by colorimetric method at 725 nm. The standard curve was prepared by 0, 5, 10, 15, 20 and 25 mg/ ml solutions of Gallic acid in methanol: water (50:50, v/v). Total phenol values are expressed in terms of Gallic acid equivalent. [18] (Siddhuraju and Becker, 2003).

2.7. Quantitative Estimation of Flavonoids

500 µl of all the plant extracts were taken in different test tubes. To each extract, 2 mL of distilled water was added. Then 150 mL of 5% $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation, 150 mL of $AlCl_3$ (10%) was added to all the test tubes including the blank. All the test tubes were incubated for 6 min at room temperature. Then 2 mL of 4% NaOH was added, which was made up to 5 mL using distilled water. The contents in all the test tubes were vortexes well, and were allowed to stand for 15 min at room temperature. The pink color developed because of the presence of flavonoids was read spectrophotometrically at 510 nm. Rutin was used for the calibration curve [19] (Zhishen et al.,

1998) and the results expressed as Rutin equivalents.

2.8. Quantitative Estimation of Terpenoids

Dried plant extract 100mg (wi) was taken and soaked in 9mL of ethanol for 24 hour [20]. The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying (wf). Ether was evaporated and the yield (%) of total terpenoids contents was measured by the formula (wi-wf/wi×100)

2.9. Quantitative Estimation of Alkaloids [21]

The quantitative estimation of alkaloid was carried for both control and urea treated plants. 1g of sample powder was dissolved in 10% acetic acid in ethanol and kept for 4 hours for each sample. Then the extracts were concentrated in water bath to one quarter of its original volume. Concentrated ammonium hydroxide is then added to the concentrated extracts for precipitation. The precipitate was collected and washed with dilute ammonium hydroxide and filtered. It is then dried, weighed and the result is expressed in percentage.

Percentage of alkaloid = (weight of alkaloid / weight of sample) × 100

2.10. Quantitative Estimation of Saponins

The quantitative estimation of saponin was carried according to the methodology of Ezeonu and Ejikeme [22] for both control and salt treated plants. 1g of sample powder was mixed with 20% of aqueous ethanol. The extracts were heated in water bath at 55°C with constant stirring for 4 hours. Then it was filtered and washed with aqueous ethanol. The mixture is then concentrated upto 4ml using water bath. The concentrated extract was then transferred into a separating funnel and mixed with 10ml diethyl ether. It was shaken well and the aqueous layer was recovered. The recovered aqueous layer was mixed with 6ml n-butanol, shaken well and the extract was recovered from it. The recovered extract was then washed twice with 10ml of 5% aqueous NaCl. The resulting solution is then heat dried, weighed and the result is expressed in percentage.

Percentage of saponin = (weight of saponin / weight of sample) × 100

The percentage of alkaloids and saponins were estimated and the range of difference is determined by comparing with control.

2.11. DPPH Radical Scavenging Activity

The hydrogen donating capacity was assessed by using stable DPPH method [23]. Briefly, a solution of 0.1mM DPPH was prepared using methanol. The samples (50- 250µg/mL) were mixed with 5.0mL of DPPH solution. Reaction mixture was shaken, incubated at 27°C for 20 min and the absorbance was measured at 517 nm. Results were compared with the activity of rutin. Percent DPPH discolouration of the sample was calculated using the formula:

DPPH radical scavenging activity (%) = [(Control OD - Sample OD) / Control OD] × 100.

3. RESULTS AND DISCUSSION

3.1. Growth of *Bacopa monnieri* under Ideal Condition

The plant grows well in wet and semi shade conditions. Stems are used to propagate. After three weeks from planting roots arose at nodes and the plant started to grow and new leaves arise at nodes. Stems showed creeping nature. The leaves formed were about 0.4 – 2.8cm long and 0.15 – 0.8 cm wide (Fig. 1).

Figure 1. *Baccopa monnieri* cultivated in different pots

3.2. Morphological Changes under Stress

The salt stress induces ionic and osmotic stress in plants leading to alterations in biochemical processes that result in morphological changes. The nitrogen containing urea when provided in excess amounts leads to several internal changes which is exhibited through morphology. Both the urea and salt treated plants showed presence of yellow coloured leaves in some stems as a result of the stress and as a mechanism to withstand it. Complete colour changes or wilting or death of the plants has

not been observed in this treatment throughout the study. Hence this study proves the concentration which is used in this study is not causes the serious effect in plant life.

3.3. Extraction Yield

The yield of plant extract in ethanol is shown in the Table 1. In urea applied plants the extract yield is higher for medium dose treated plant, which is 5.77% and the control plant having low yield of 5.08%. In NaCl applied plants high yield of extract got in control plant which is 5.71% and medium dose applied plant having low yield of 5.22% (Table 1).

Table 1. Extraction yield of *Bacopa monnieri*

Concentration of urea applied (mg)	Extract yield (%)	Concentration of NaCl applied (mM)	Extract yield (%)
Lower dose (250 mg)	5.08	Lower dose (100 mM)	**5.71**
Medium dose (500 mg)	**5.77**	Medium dose (300 mM)	5.22
Higher dose (1000 mg)	5.17	Higher dose (400 mM)	5.70

Lower dose- normal requirement of the plant; Medium dose and higher dose- stress applied doses

3.4. Quantitative Estimation of Total Phenolic Contents

The contents of total phenols present in both plants kept as control and those treated with NaCl that were measured by Folin Ciocalteu reagent in terms of gallic acid equivalent. The total phenol varied from 1812.25 to 2057.125 µg GE/mg. The result showed that more amount of phenolics were synthesized in those plants treated with 400mM NaCl/Kg of soil when compared to that of plants kept as lower dose stress treatment (Table 2).

Stress caused by NaCl induced an accumulation of proline, total phenolics and other antioxidants in rosemary (*Rosmarinus officinalis*) [24]. The total phenolic content of sprouts of radish treated with 100 mM of NaCl was significantly increased, which is similar to that of *Cakile maritima* and red pepper reported by Ksouri et al. [25]. The quantity of total phenols was observed to be 24.75 mg GAE/gm methanolic extract of *Bacopa* [26].

Table 2. Quantitative estimation of total phenolic and saponin contents

Concentration of NaCl applied (mM)	Phenolics µg GE/mg	Saponin (%)
Lower dose (100 mM)	1812.25±0.10	12.23%
Medium dose (300 mM)	1915.54±0.035	17.2%
Higher dose (400 mM)	**2057.125±0.05**	**25.56%**

Values are mean ± Standard Deviation
Lower dose- normal requirement of the plant; Medium dose and higher dose- stress applied doses

3.5. Quantitative Estimation of Saponins

The saponin content present in both plants kept as control and those treated with NaCl is shown in Table 2. It represents the saponin of the control is 12.23%, it was exceeded for the salt treatment plants 300mM and 400mM as 17.25 and 25.56% respectively. The result showed that more amount of saponins were synthesized in those plants treated with 400mM NaCl when compared to that of plants kept as control and lower dose stress treatment.

In the phytochemical analysis of *Bacopa monnieri* the quantitative estimation of saponins (mg/g dry weight) has showed that root contains 7.50±0.71, stem contains 23.33±1.53 and leaf contains 57.00±2.65 saponins [27]. In another phytochemical study the quantitative analysis of saponins in the whole plant was 1.5 mg/g dry matter of plant extract [26]. The phytochemical studies upon the plant after growing under salinity and drought stress in *in-vitro* condition has induced increase in proline content more than 20 times than that of the control [28]. In another study of growing the plant in salt stressed conditions by applying halotolerant rhizobacteria showed higher levels of proline and biomass yield [29]. So the increase in synthesis of secondary metabolites can be achieved through stress application which opens a wider scope in the near future.

3.6. Quantitative Estimation of Flavonoids, Terpenoid Content

The flavonoid content present in both plant kept as control and those treated with urea is shown in Table 3. The flavonoid varied from 95.68 to 450.72 µg RE/mg. The result showed that more amount of flavanoids were synthesized in those plants treated with 500mg urea/Kg soil when compared to that of plants kept as lower and higher dose stress treatment. The higher dose of urea (1000mg/kg soil) synthesized only 161.25 µg RE/mg; it seems the stress applied with higher concentration of urea affects the flavonoid production. Increases in flavonoids under low N supply might also be attributed to promoting deamination of phenylalanine to cinnamic acid due to nitrogen deficiency [30]. The flavonoids in tomato, Arabidopsis and alfalfa root decreased with increasing N supply [31]. Total flavonoid content of *Bacopa monnieri* (L.) at 10 µg was recorded as 6.333 GAE and at 1000 µg it increased to 29.666GAE [26].

The terpenoid content present in both plant kept as control and those treated with urea is shown in Table 3. It is revealed from the result that the control plant gives 15% terpenoids which is lesser than the 100 mg/kg urea treated which is 28% and 200 mg/kg urea treated which is 36%. The result showed that the more amount of terpenoids were synthesized in those plants treated with 200 mg/kg of soil when compared to that of plants kept as control and lower dose stress treatment. Under moderate nutrient soil concentrations, N and P do not limit terpenoid production, because plants may take up enough N and P to fulfil their requirements for growth and terpenoid synthesis [32]. The concentrations of most of the terpenoids identified in the plant material were significantly influenced by N supply, yet the response varied between different groups of terpenoids [33]. *Bacopa monnieri* (L) was reported to possess terpenoids and steroids predominately in ethanol, aqueous, chloroform, acetone and ethyl acetate extracts [34].

3.7. Quantitative Estimation of Alkaloids

The alkaloid content present in both plants kept as control and those treated with urea is shown in Table 3. The data being responsible for that the control plants gives 22.2% alkaloids which is lesser than the 100mg/kg urea treated which is 49.4% and 200 mg/kg urea treated which is 71.4%. The results showed that more amount of alkaloids were synthesized in those plants treated with 200 mg urea/kg of soil when compared to that of plants kept as control and lower dose stress treatment.

In a scientific study, the phytochemical analysis of *Bacopa monnieri* provides the quantitative estimation of alkaloids (mg/g dried weight) has showed that root contains 1.67±0.577, stem contains 47.00±0.81 and leaf contains 53.07±2.08 alkaloids [27]. In another phytochemical study the quantitative analysis of alkaloids in the whole plant was 110mg/g dry matter of plant extract [26]. It was revealed that the quantity of alkaloids was enhanced in *Catharanthus roseus* through the effect of nitrogen fertilization when compared with its parental variety [16]. Thus the study revealed that a good amount of alkaloid can be enhanced through stress application by various biotic and abiotic factors which has a lot of scope in the pharmaceutical industry.

Table 3. Quantitative estimation of flavonoids, terpenoid and alkaloid content

Concentration of urea applied (mg)	Flavonoids µg RE/mg	Terpenoids (%)	Alkaloids (%)
Lower dose (250 mg) Control	95.68±0.14	15	22.2%
Medium dose (500 mg)	**450.72±0.56**	28	49.4%
Higher dose (1000 mg)	161.25±0.47	**36**	71.4%

Values are mean ± Standard Deviation
Lower dose- normal requirement of the plant; Medium dose and higher dose- stress applied doses

3.8. DPPH Radical Scavenging Activity

Antioxidants through their scavenging power are useful for the management of these diseases. DPPH stable free radical scavenging method is an easy, rapid and sensitive way to survey the antioxidant activity of a specific compound or plant extracts [23].

The antioxidant activity is increased in urea treated plants and there is decrease of antioxidant activity in NaCl treated plants. In urea treated plants, the control plants show minimum antioxidant activity of 11.91±0.547, while 1g/kg urea treated plants show high activity (19.97±0.039 %) as compared to other two dose (Table 4). In NaCl applied plants, the control plants show high antioxidant activity of 22.32±1.6 as compared to two other doses. Though the phenolic compounds are much increased in higher dose of NaCl stress application, the antioxidant activity has reduced. It shows other metabolites interfere the radical scavenging activity of the plant extract. It should be focused and traced to find the active and inhibitory metabolites of *B. monnieri* through further research works.

Table 4. Determination of antioxidant activity through DPPH radical scavenging activity

Concentration of NaCl Stress applied (mM)	DPPH radical inhibition (%)@ 60µg of extract	Concentration of Urea stress applied (mg)	DPPH radical inhibition (%) @ 60µg of extract
Lower dose (100 mM)	**22.32±1.6**	**Lower dose (250 mg)**	11.91±0.54
Medium dose (300 mM)	16.10±1.49	**Medium dose (500 mg)**	15.40±0.49
Higher dose (400 mM)	8.64±0.99	**Higher dose (1000 mg)**	**19.97±0.039**

Values are mean ± Standard Deviation
Lower dose- normal requirement of the plant; Medium dose and higher dose- stress applied doses

In another research study of *Mitracarpus scaber* the phytochemical screening of crude methanolic extract revealed the presence of saponins, tannins, flavonoids, essential oil and glycosides. The antioxidant activity using DPPH analysis shows that the high antioxidant capacity [35]. Methanolic extracts of *Chromolaena odorata* showed higher free radical scavenging capacity and phytochemical analysis revealed the presence of tannins, phlorotannins, steroids, terpenoids, flavonoids and saponins [36]. Non-enzymatic antioxidants like cysteine, non-protein thiol, proline, carotenoids and ascorbic acid may play a role in inducing resistance to salinity by protecting labile macromolecules against attack by free radicals which are formed during various metabolic reactions leading to oxidative stress [37, 38].Among the eight different concentrations (20 to 800µg/ml) of ascorbic acid and leaf extracts of two plant sources, the highest scavenging activities were 96.86%, 75.85% and 86.02%, respectively for ascorbic acid, *B. monniera* and *C. grandis* at 800µg/ml concentration with similar extract of the aerial parts of *B. monnieri* also noted concentration dependent scavenging activities [39].

Plant medicinal properties are well known and many are yet to be discovered. Almost all the parts of the plant namely stem, leaves, flowers, fruits, roots and seeds are known to have medicinal properties based on the chemical components present in it. These parts can be used to extract medicinally important compounds. They can be utilized to cure several diseases and lead a better life. The medicinal properties of plants have greatly contributed to the pharmaceutical industry. There are several herbal therapies available to treat several ailments.

4. CONCLUSION

The higher amount of saponins, phenolics produced in plants treated with NaCl. A higher dose of urea increased the production of flavonoids and terpenoids than alkaloids. The antioxidant activity is increased in urea treated plants and there is decrease of antioxidant activity in NaCl treated plants. The study proves that there is production of secondary metabolites during stress conditions in the plant *Bacopa monnieri*. Production is higher in such treated plants when compared to plants kept as controls. This piece of research has paved a way for its improved commercial production of attested compounds/drug. Further, the researchers can also proceed with other research analysis based on compounds synthesized through the application of different stresses to this plant.

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REFERENCES

1. Singh, R. and Geethanjali (2018). Chemotaxonomy of Medicinal Plants: Possibilities and Limitations. *Natural products and drug discovery,* 119 - 136.
2. Bhattacharjee, T., Sen S., Chakraborty, R. and Maurya, P. K. (2019). Cultivation of Medicinal Plants: Special Reference to Important Medicinal Plants of India. *Herbal Medicine in India* 101-115.
3. Verpoorte, R. (2005). Encyclopedia of Analytical Science. Elsevier; Amsterdam, the Netherlands. In *Alkaloids* 56–61.
4. Heinrich, M., Mah, J. and Amirkia, V. (2021). Alkaloids Used as Medicines: Structural Phytochemistry Meets Biodiversity-An Update and Forward Look. *Molecules* 26(7):1836.
5. Zhang, H., Zhao, Y. and Zhu, J. K. (2020). Thriving under stress: how plants balance growth and the stress response. *Developmental Cell* 55(5): 529-543.
6. Pooja, A. S. and Sharma, J. (2020). Stress Physiology in Plants. Reforms in Agriculture and Rural Development under Covid-19 Pandemic. *Society of Human Resource and Innovation* (812 Paschimpuri, Agra-282007 (U.P.) at M/S Shree Krishna Publishers, AGRA (U.P.) 175-186.
7. Mahajan, M., Kuiry, R. and Pal, P. K. (2020). Understanding the consequence of environmental stress for accumulation of secondary metabolites in medicinal and aromatic plants. *Journal of Applied Research on Medicinal and Aromatic Plants* 18, 100255.
8. Mahajan, S. and Tuteja, N. (2005). Cold, salinity and drought stresses: An overview. *Arch Biochem Biophys* 444,139-58.
9. Srivastava, S. and Srivastava, A. K. (2013). Biotechnology and Genetic Engineering for Alkaloid Production. In: Ramawat K.G., Mérillon J.-M., editors. Natural Products:

Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes. Springer; Berlin/Heidelberg, Germany. 213–250.
10. Yu, B. W., Chen, J. Y., Wang, Y. P., Cheng, K. F., Li, X. Y. and Qin, G. W. (2002). Alkaloids from *Menispermum dauricum*. *Phytochemistry* 61(4), 439-442.
11. Ashour, A. S., Abed El Aziz, M. M. and Al Sadek, G. M. (2019). A review on saponins from medicinal plants: chemistry, isolation, and determination. *Nanomedicine Research* 7(4), 282-288.
12. Ramakrishna, R. and Ravishankar, G. A. (2011). Influence of abiotic stress signals on secondary metabolites in plants. *Plant Signal Behaviour* 6(11), 1720-1731.
13. Isah, T. (2019). Stress and defense responses in plant secondary metabolites production. *Biological Research* 52(39), 1-25.
14. Hassen, S., Sheeba, F., Saba, A., Rahman, A., Iffat, Z. A. and Saeed, M. (2012). Current approaches toward production of secondary plant metabolites. *Journal of Pharmacy and Bioallied Sciences* 4(1), 10-20.
15. Abdel-Farid, I. H., Marghany, M. R., Rowezek, M. M. and Gabr, S. M. (2020). Effect of Salinity Stress on Growth and Metabolomic Profiling of *Curcumis sativus* and *Solanum lycopersicum. Plants* 9(11), 1626.
16. Sreevalli, Y., Kulkarni, R. N. and Kushik, B. (2004). Increasing the content of leaf and root alkaloid content mutants of periwinkle through nitrogen fertilization. *Industrialization Crops and Products* 19(2), 191-195.
17. Sivaramy, R. E. (2019). Bacopa Monnieri - A Review. *International Journal of Trend in Scientific Research and Development* 3(2), 503 – 507.
18. Siddhuraju, P. and Becker, K. (2003). Studies on antioxidant activities of Mucuna seed (*Mucuna pruriens* var. utilis) extracts and certain non-protein amino/imino acids through in vitro models. *Journal of the Science of Food and Agriculture* 83, 1517–1524.
19. Zhishen, J., Mengecheng, T. and Jianming, W. (1999). The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. *Food Chemistry* 64, 555–559.
20. Indumathi, C. G., Durgadevi, S., Nithyavani and Gayathri P. K. (2014). Estimation of terpenoid content and its antimicrobial property in *Enicostemma litorrale*. *International Journal of ChemTech Research* 6 (9), 4264 -4267.
21. Harborne, J. B. (1976). Phytochemical Methods: A guide to Modern Techniques of Plant Analysis. Published by Chapman and Hall. 1-278.
22. Ezeonu, C. S. and Ejikeme, C. M. (2016). Qualitative and Quantitative determination of phytochemical contents of indigenous Nigerian Softwoods. *New journal of science*. 2016,1-9.
23. Blois, M. S. (1958). Antioxidants determination by the use of a stable free radical. *Nature* 4617, 1199-1200.
24. Zheng, W. and Wang, S.Y. (2001). Antioxidant activity and phenolic compounds in selected herbs. *Journal of Agricultural and Food Chemistry* 49, 5165–5170.
25. Ksouri, R., Megdiche, W., Debez. A., Falleh, H., Grignon, C. and Abdelly, C. (2007). Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte *Cakile maritima*. *Plant Physiology and Biochemistry* 45(3–4), 244–249.
26. Jain, P., Sharma, H. P., Basri, F., Priya, K. and Pallavi Singh. (2017). Phytochemical analysis of *Bacopa monnieri* (L.) Wettst. and their antifungal activities. *Indian Journal of Traditional Knowledge* 16(2), 310-318.
27. Anju, V., Naresh, C. and Avinash, P. (2017). Anatomical markers and Phytochemical study of different plant parts of *Bacopa monnieri* (L.) Wettst. *International Journal of Life Sciences* 5(3), 379-386.
28. Debnath, M. (2008). Responses of *Bacopa monnieri* to salinity and drought stress in vitro. *Journal of Medicinal Plants Research* 2(11), 347-351.
29. Bharti, N., Deepthi, Y., Deepthi, B., Deepamala, M. and Alok, K. (2013). *Exiguobacterium oxidotolerants*, a halotolerant plant growth promoting rhizobacteria, improves yield and content of secondary metabolites in *Bacopa monnieri* (L.) Pennel under primary and secondary salt stress. *World Journal of Microbiology and Biotechnology* 29, 379-387.
30. Bongue-Bartelsman, M. and D. A. Phillips. (1995). Nitrogen stress regulates gene

expression of enzymes in the flavonoid biosynthetic pathway of tomato. *Plant Physiology and Biochemistry* 33, 539-546.
31. Kondorosi, A. P., Ratet and C. Coronado. (1995). Alfalfa root flavonoid production is nitrogen regulated. *Plant Physiology* 108, 533–542.
32. Blanch, J.S., Peñuelas, J., Sardans, J. and Llusià, J. (2009). Drought, warming and soil fertilization effects on leaf volatile terpene concentrations in *Pinus halepensis* and *Quercus ilex*. *Acta Physiologiae Plantarum* 31, 207-218.
33. Saloner, A. and Bernstein, N. (2021). Nitrogen supply affects cannabinoid and terpenoid profile in medical cannabis (*Cannabis sativa* L.). *Industrial Crops and Products* 167,113516.
34. Subashri, B. and Justin, Y. K. P. (2014). A comparative study of antioxidant activity of *Baccopa monnieri* (l.) Pennell using various solvent extracts and its GC-MS analysis. *International Journal of Pharmacy and Pharmaceutical Sciences* 6(2), 494-498.
35. Ali, M. N., Ndahi, J. A., Abdullahi, A. and Yelwa, J. M. (2021). Phytochemicals, chemical composition and antioxidants profile of the crude extracts and essential oil of *Mitracarpus scaber* (Goga masu) Phytochemicals, chemical composition and antioxidants profile of the crude extracts and essential oil of Mitracarpu. *Journal of Research in Chemistry* 2(2), 27-31.
36. Akinmoladum, T., Kajikawa, I., Nishiya, K., Takeya, K. and Itokawa. H. (2007). Studies on the constituents of Japanese mistletoes from different host trees, and their antimicrobial and hypotensive properties. *Chemical and Pharmaceutical Bulletin* 37(6), 1543-1546.
37. Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry* 27, 969–978.
38. Galli, U., Schuepp, H. and Brunold, G. (1996). Thiols in cadmium and copper treated maize (*Zea mays* L.). *Planta* 198, 139-143
39. Ghosh, T., Maity, T. K., Das, M., Bose, A. and Dash, D. K. (2007). *In vitro* antioxidant and hepatoprotective activity of ethanolic extract of *Bacopa monniera* L. aerial parts. *Iranian Journal of Pharmacy & Therapeutics* 6(1), 77-85.

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