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PHYTOCHEMICAL ANALYSIS AND CHLOROPHYLL CONTENT UNDER VARIOUS CONCENTRATION OF SALT SALINITY IN MORINGA OLEIFERA LAM

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ABSTRACT

Screening of phytochemical is an expensive step in the detection of bioactive principal compounds present in the several plants that may leads to novel drug investigation. *Moringa oleifera* leaves are an excellent source of vitamins (especially Vitamin A, B and C), minerals (calcium, iron) and protein. They are used to combat malnutrition, blindness, diabetes, high blood pressure, anemia, urinary tract problems, kidney stones, to induce lactation in nursing women, and as an antiseptic. In present the study leaf of *M. oleifera* plant have been selected for phytochemical screening and chlorophyll a and b content under different concentration of sodium chloride. The level of salt stress was introduced control (0%), 0.5%, 1%, 1.5% and 2% for 40 days The plants were grown in triplicate.

KEYWORDS: Salinity stress, phytochemical screening, chl a and b, *Moringa oleifera*.

INTRODUCTION

The medicinal plants are useful for therapeutic as well as curing of human disease because of presence of several phytochemical constituents.[1] Plants have been considered basis of traditional medicine practices used for thousands of years by people of China, India and many other countries.[2] Earlier record suggested the usage of plant in the Atharveda, which is the best Ayurvedic medicine in India. The plant chemicals are called natural product or secondary metabolites synthesized in all parts of the plant body, bark, leaves, stem, root, flower, fruits, seeds etc. Most of the plant part synthesized active component.[3] They are synthesized variety of chemical substances including alkaloids, steroids, flavonoids,

terpenoids, glycoside, saponia, tannins and phenolic compounds.[4] Medicinal plants are considered as therapeutic effective and safer alternatives to the synthetic antibiotics.[5] The plant *Moringa oleifera Lam.* belongs to the family *Moringa*cae and is a native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan where it is used in folk medicine.[6] *M. oleifera*is referred to as a "miracle tree" or a "wonder tree"[7] of significant socio economic importance because of its several nutritional, pharmacological.[7,8] It is deciduous tree or shrub, fast growing, drought resistance, average height of 12 meter at maturity. All parts of the *Moringa* tree are edible and have long been consumed by humans.[6] Its leaves have the calcium equivalent of four times that of milk, the vitamin C content is seven times that of orange while its potassium is three times that of bananas, three times the iron of spinach, four time, the amount of vitamin A carrots, and two time the protein in milk.[9]

MATERIAL AND METHOD

Experimental material and salt treatments: Pods of drumstick (*Moringa oleifera* Lam.) collected from premises of Late Pundalikrao Gawali Arts and Science Mahavidyalaya, Shirpur (Jain), District- Washim, (M.S.) brought it in the laboratory of Botany Department for identification. The plant material was identified by using standard floras like.[10] The seeds were taken out of the pod. Ten healthy seeds of uniform size were selected for the experiment. Seeds surface were sterilized in 70% (v/v) ethanol for 2 min and 3% sodium hypochlorite solution for 20 min followed by washings for several times with distilled water. The seeds have been shown in plastic pot of diameter 10x 10 cm in size containing 250 g soil at constant temperature of 25°C. The deionized water was given regularly a day after interval up to 15 days. The seedling attained maximum height. Laterseedlings were introduced 0%, 0.5%, 1%, 1.5% and 2% Na Cl treatment. Each treatment was replicated three times and pots were randomly arranged during the growth period. Seedling was kept in controlled environmental condition up to 40 days. The leaves were cut forthe phytochemical screening.

Phytochemical Analysis: For the phytochemical analysis of following Phytochemicals, leaves were extracts with 90% ethanol of above mentioned plants.

Preliminary photochemistry

The preliminary phytochemical study was done for detection of various constituents i.e. alkaloids, glycosides, carbohydrates etc. present in leaf extract, which is responsible for the

pharmacological activity. Chemical tests were carried out on the successive extracts separately using standard procedures to identify the constituents as described by.[11,13]

Test for Alkaloid (Mayer Test): Weigh about 0.2 gm of plant extract in a test tube and warmed with 2% sulphuric acid for 2 min., and add few drops of mayerreagent and observed for the presence of orange red precipitate for the presence of alkaloid.

Test for Flavanoid (Shinoda Test): To 1ml of the extract, add 8 - 10 drops of concentrate HCl and a pinch of magnesium powder or filing. Boil for 10 to 15 minutes and cool. A red colouration indicates the presence of flavonoids.

Test for Cardiac Glycosides (Keller-Killani test): To 5ml of the extract is mixed with 2ml of glacial acetic acid containing one drop of ferric chloride solution and 1ml of concentrated sulphuric acid. A brown ring at the interface indicates the presence of cardiac glycosides.

Test for Terpenoids (**Salkowski test):** Take 5ml of the extract add 2ml of chloroform and 3ml of concentrated H2SO4. Formation of yellow colour ring at the interface of the two liquids that turns reddish brown colour after two minutes, showed the presence of terpenoids.

Test for Phenols (**Liebermann's test):** Take 1ml leaf extract add 1ml of sodium nitrite, few drops of diluted sulphuric acid and 2ml of diluted NaOH. Appearance of deep red or green or blue colour indicates presence of phenol.

Test for saponins (Froth Test): 2gm of sample was added in 10 ml of distilled water and shaken well. Froth formation confirms the presence of saponins.

Test for steroids: Take the plant extract add 2ml of acetic anhydride and add 0.5 gm of ethanol with 2ml of sulphuric acid. Violet to blue or green colour indicates the presence of steroids.

Test for Protein: Add few drops of Millon's Reagent to 2 ml of plant extract. White coloured precipitate confirms the presence of Proteins.

Test for Amino acid (Ninhydrin Test): Take 1 ml leaf extract in a test tube heat it for 2 min, add 10% ninhydrine in it after some time violet coloured is formed indicated the presence of amino acid.

Table 1: Phytochemical screening of *M. oleifera* leaves.

Sr. No.	% Na Cl Treatment	Phytochemical test 90% Ethanol extract									
		AL	FL	ST	CAR GLY	TER	PH	TAN	SAP	PRO	AA
1	Control (0%)	+	+	+	+	+	+	+	+	+	+
2	0.5	+	+	+	+	+	+	+	+	+	+
3	1	+	+	+	+	+	+	+	+	+	+
4	1.5	+	-	+	-	+	+	-	+	+	+
5	2	-	-	+	-	+	-	-	+	+	+

Note: (+) Indicate presence of phytochemical, (-) Indicate absence of Phytochemical.

Abbreviation- AL: Alkaloid, **FL:** Flavonoid, **ST:** Steroid, **CAR GLY:** Cardiac glycoside, **TER:** Terpenoids, **PH**: Phenol, **TAN:** Tannin, **SAP:** Saponin, **PRO:** Protein, **AA:** Amino acid.

Chlorophyll Estimation

Chlorophyll content of *Moringa olifera* Lam. leaf of different concentration level was analyzed by method reported by.[14] Each of the samples was weighed 100 mg and homogenized separately in a mortar in the presence of excess of 80% acetone until all the color was released from the tissue. The samples were then centrifuged at 5000 rpm for 10 minutes at room temperature. The clear supernatant was collected and then made up to a known volume (5 ml). Immediately it was used for recording of absorbance using spectrophotometer (ECI, India) and was adjusted at wavelength of 663 nm for chlorophyll ‘a’ and 645 nm for chlorophyll ‘b’ set at 100% transmittance using 80% acetone as blank before taking the readings of the samples respectively. The optical density was measured and the chlorophyll contents in the original extract was estimated using the formula

$$\text{Chl a} = \frac{(12.7\ A663)-(2.63\ A645)}{\text{Weight (g)}} \times 1000$$

$$\text{Chl b} = \frac{(22.9\ A645)-(4.48\ A663)}{\text{Weight (g)}} \times 1000$$

Table 2: The effect of salt salinity on chlorophyll pigment of *M. oleifera*.

Sr. No.	Na Cl %	Chl a (ug/ ml)	Chl b (ug/ ml)	Chl a/ b ratio
1	Control	3.637	2.638	1.37
2	0.5%	3.292	2.438	1.35
3	1%	3.077	2.301	1.33
4	1.5%	2.650	2.252	1.17
5	2%	2.214	1.751	1.26

RESULT AND DISCUSSION

The preliminary phytochemical screening of leaf extract in 90% ethanol was done for the presence or absence of bioactive compound. Alkaloid is seen in all treatment but absent in 2% NaCl. Flavonoid, tannin and cadiac glycoside occurred in control, 0.5%, 1% but absent 1.5% and 2% in NaCl respectively. Steroid, terpenoids, saponin, protein and amino acid present in all concentration of NaCl.

The effect of salt salinity on chlorophyll pigment shows that there is significant decrease in chlorophyll pigment. Higher salinity reduced the chlorophyll b content at 2% NaCl concentration while chlorophyll a is also affected by various concentration of salinity. Similar result was found in spinach exposed to salinity stress.[15] The ratio of chlorophylla/ b in control, 0.5%, 1%, 1.5% and 2% found to be 1.37, 1.35, 1.33, 1.17 and 1.26 respectively.

CONCLUSION

Based on present result, it is concluded that *M. oleifera* can be cultivated in salt affected region might increase in production of secondary metabolites at plant level while chlorophyll pigment synthesis shows negative effect. Nonetheless a detailed investigation regarding to this suitability of plant to varying salinity level from low to high in addition to detailed field experiment needed to confirm this conclusion.

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