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Allelopathic Potential of the Purified Fraction MN-F10 from Nyctanthes arbor-tristis L. on Early Seed Growth and Seedling Characteristics of Vigna radiata (L) Wilczek

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Abstract: **-- The plants are the reservoirs of structurally different bioactive molecules. The isolation and identification of phytochemicals from plants is crucial for discovery of new bioactive compounds to be used by various industries. Our previous study has reported the strong allelopathic potential of methanolic leaf extract of Nyctanthes arbor-tristis L. for the first time (Antony S and P.J. Benny, 2017). This study highlighted the characterization of an allelochemical present in the purified fraction, MN – F10 from Nyctanthes arbor-tristis L. through various spectroscopic assays. The potential allelopathic effect of MN - F10 was identified by assessing a dose dependent reduction in the various growth characteristics of Vigna radiata (green gram) seedlings including early seedling growth, length of root and shoot, seedling vigor index and fresh & dry weight. All these growth parameters reduced at the same rate. This study warrants further investigation of allelopathic potential of MN-F10 in field condition, which will finally leads to the development of a potential bioherbicide.**

Keywords **– Allelopathy, Nyctanthes arbor-tristis, Spectral analysis, Vigna radiate.**

I. INTRODUCTION

Allelochemicals are phytochemicals inhibiting the growth and development of neighbouring living organisms. (Reese, 1979). The exploration of different allelochemicals from various natural sources especially from plants leads to the production of new generation of bioherbicides and biopesticides, which alleviates the problems of their chemical counterparts (Islam and Kato-Noguchi, 2012). Various researchers has already reported the allelopathic potential of several plants. (Javaid A. and Shaflquel, 2006, Khan et al, 2001). Studies from our group has reported the allelopathic potential of methanolic leaf extract of Nyctanthes arbor-tristis L. for the first time (Antony S and P.J. Benny, year). Nyctanthes arbor-tristis L. is a popular garden plant known in India since antiquity, which is having several biological properties and also used for the development of some industrial products (Kirtikar and Basu, 1918) Several biological properties of the plant has been reported earlier, which includes antihistaminic, CNS, analgesic, anti-inflammatory, antipyretic, antiulcer, amoebicidal, anthelmintic, antitrypanosomal, antidepressant, antiviral and immunomodulatory activities (Sasmal et al., 2007). Phytochemical analyses of the plant, Nyctanthes arbor-tristis L. has revealed the presence of several compounds including phytosterols, phenolics, tannins, alkaloids, anthocyanin, carotenoids, flavonoids, glycosides, B-sitosterol, oleanolic acid and saponins (Khatune et al., 2003; Omkar et al., 2006 and Iyer et al., 1998). The present study has investigated the herbicidal activity of the purified fraction isolated from the methanolic leaf extract of Nyctanthes arbor–tristis on early seed and seedling growth parameters of Vigna radiata. Our attempt to purify the active extract leads to the identification of a compound and we presume that it may be the active compound responsible for the allelopathic activity of the plant.

II. MATERIALS AND METHODS

Purification of the methanolic extract

Purification was performed using bioactivity guided fractionation. All the chemicals used for column and thin layer chromatography were purchased from Merck, India. The methanolic crude extract was fractionated using silica gel column chromatography, in which the extract was eluted successively with 100% chloroform, linear gradients of chloroform /methanol and finally with 100% methanol respectively. A total of thirty fractions (30 ml/fraction) were collected separately and concentrated by vacuum evaporation. The allelopathic activity of all these fractions was tested by using seeds of Vigna radiata due to its easy availability, ease of handling and easy germination. Fractions that showed allelopathic activity were tested for

purity by TLC. The successive active fractions showing similar banding pattern on the TLC chromatogram were pooled and again subjected to stepwise gradient elution with chloroform methanol mixture. This procedure was repeated until a pure active principle was isolated from the extract, which was named as MN F-10.

Determination of allelopathic potential of MN F-10

Four different concentrations of MN F-10 (1, 1.5, 2, and 2.5mg/ml respectively) were applied to petriplate containing fixed number of Vigna radiata seeds. A control plate was also maintained in the similar way to compare the results. After one week, ten seedlings from both control and treatment plates were randomly selected for recording morphological parameters such as seedling growth (root and shoot lengths) and biomass production (fresh and dry weight) to determine the efficacy of this fraction. The seed germination percentage and seedling vigor index were also determined. Three replicates were performed.

Root length and Shoot length

Root length and shoot length of the treated seedlings were measured and compared with that of control.

Fresh weight and dry weight of seedlings

After washing with distilled water, the fresh weight of seedlings was recorded. Then they were kept in a hot air oven at 80ºC for 24 hours and the dry weight was also taken.

Germination percentage

After the specified period, the number of germinated seeds was counted and germination percentage was calculated by using the following formula (Vaithiyanathan, 2014).

$$\text{Gemination Percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

Seedling vigor index

Seedling vigour index was calculated by the following formula,

Vigour Index = Root length + shoot length × seed germination percentage (Maisuria & Patel 2009).

Characterization of MN F-10

The purity of MN F-10 was checked by TLC, which gives a single spot with chloroform / methanol [80:20]. The purified fraction was later characterized using different spectral analyses (UV – VIS, FT- IR, 1H NMR and 13C NMR) for its structural elucidation.

III. RESULTS AND DISCUSSION

The growth inhibitory activity of the leaf extract was further confirmed after determining the allelopathic activity of MN F-10 isolated from the same extract. MN F-10 was tested towards various parameters in order to prove its allelopathic potential, which includes the percentage of seed germination, root length, shoot length & fresh and dry weight of seedlings as well as the seedling vigor index of Vigna radiata. MN F-10 has displayed a concentration dependent reduction in all the above parameters, revealing its significant allelopathic activity towards Vigna radiata ***(Table: 1).***

Parameters studied	Test Concentrations (mg/ml)				
	0	**1**	**1.5**	**2**	**2.5**
G_T	100.00± 0.00	92.12± 0.05	88.24± 1.21	40.05± 1.89	0.00±0. 00
S L (cm)	11.1±2. 14	6.8±1.9 8	2.9±1.8 5	1.27±1. 10	0.021± 0.04
RL (cm)	4.9±1.1 2	2.44±0. 05	1.12±0. 21	0.75±0. 02	0.00±0. 00
FW(g/pl ant)	1.97±0. 07	1.62±0. 54	1.081± 0.00	0.665± 0.00	0.320± 0.00
DW(g/pl ant)	0.431±0 .21	0.294± 0.04	0.201± 0.00	0.09±0. 00	0.030± 0.00
SVI	1157.3± 1.25	319.28 ±1.4	188.46 ±1.4	60±1.2 5	0.00±0. 21

Table 1: Effect of different concentrations of MN F-10 on morphological parameters of Vigna radiata seedlings. GT represents percentage of seed germination, SL represents shoot length, RL represents root length, FW represents fresh weight, DW represents dry weight, SVI represents Seedling Vigour Index. Values are expressed as mean ± SD.

MN F-10 was further subjected to different spectral analyses to characterize the fraction and to identify the active principle/compound present in it. The results of the spectral analyses proved the presence of a compound "4-Nitrophenyl(2S)-3-{[(2R,3R,4R,5S,6R)-3-acetamido-4,5-diacetoxy-6(acetoxymethyl)tetrahydro-2H-pyran-2-yl]oxy}-2- aminopropanoate" in the purified fraction [Fig:1].

In the UV – VIS spectrum, a peak was observed at 231.00 nm. This may be due to the presence of $\pi \rightarrow \pi^*$ transition of carbonyl group. At 276nm, a relatively weak signal was observed, which represents the $n \rightarrow \pi^*$ transition of carbonyl group, (a symmetry forbidden transition).

The IR spectrum showed a broad band centered at 3424.89 cm-1 which indicates the presence of intermolecular hydrogen bonded N-H stretching vibrations. Peaks at 2955.78 cm-1& 2923.05 cm-1 indicates aromatic C-H stretching and aliphatic C-H stretching vibrations respectively. The peak at 1715.17 cm-1 arise from C=O stretching vibrations. The signals at 1633.24 cm-1&

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1151.46 cm-1 indicates the presence of C=C and N=O stretching vibrations respectively. A peak at 1449.51 cm-1shows C-H bending vibrations and another peak at 1373.40 cm-1shows C-N stretching vibrations. The signals at 1281.06 cm-1& 1159.60 cm-1 arises from C-C stretching vibrations and C-O stretching vibrations respectively. In the proton NMR spectrum a signal observed at 8.021ppm which shows the presence of amide protons which supports the proposed structure. The multiplet centred at 7.468 ppm clearly indicates the presence of aromatic protons. The peak at 5.367 ppm shows the presence of N-H protons of amino group. A peak at 4.149 ppm indicates the presence of –CH-O group. The region between 3 ppm to 4 ppm shows six signals. Among these, the peaks at 3.847, 3.689, 3.568 ppm and 3.440 ppm indicates the presence of – COOCH3, CH2-O, COCH3, and CH-NH2 respectively. The signals at 3.409 ppm & 3.295 ppm show the presence of COCH3 group. A multiplet at 1.258 ppm represents NHCOCH3 group. The peak at 7.30 ppm corresponds to the solvent (Chloroform) protons. The 13 C NMR Spectrum shows a strong signal at 132 ppm, which may be due to the C=C carbon and 168 ppm which corresponds to a aromatic group. Peaks are obtained at 61.425 ppm, 51.876 ppm and 30.055 ppm, which indicate the presence of COOCH3, CO-CH3, and NHCO-CH3 respectively.

Since"4-Nitrophenyl(2S)-3-{[(2R,3R,4R,5S,6R)-3-acetamido-4,5-diacetoxy-6(acetoxymethyl)tetrahydro-2H-pyran-2-yl]oxy}-2- aminopropanoate" is the major compound present in the purified fraction, we suggest that the allelopathic potential of MN F-10 may be correlated to the activity of this compound. However a more detailed investigation is needed to confirm this observation including the total chemical synthesis of the compound, its activity determination and its comparison with the isolated compound.

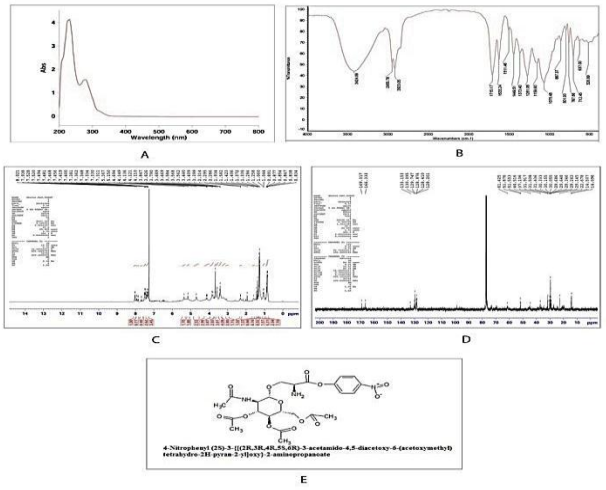

Fig. 1: Spectral analyses of MN - F10 isolated from Nyctanthes arbor – tristis L. (A) UV-VIS spectrum, (B) FT-IR spectrum, (C) 1H NMR spectrum, (D) 13C NMR spectrum, (E) structure of the proposed compound.

Herbicidal action of several other plants towards different Vigna species has been reported earlier. The seed germination inhibitory activity of Tectona grandis L. leaf extracts was reported on Vigna radiata (L.) and Capsicum frutescens L. (Leela and Arumugam, 2014).The aqueous extract of Typha angustifolia L. displayed its herbicidal potential towards Vigna mungo L. (Sethuraman and Sanjayan, 2013).The present study observed a significant inhibition of the radicle and plumule length of purified fraction treated seedlings when compared to their untreated counterparts. Several previous studies have also revealed that allelochemicals were more sensitive to root and shoot growth (Jadhar and Gayanar,1992; Manikandan and Jayakumar, 2012 and Jarchow and Cook, 2009).

The reduction in root growth might have contributed to the decline in the biomass of the seedlings, as it is adversely affect various vital functions of the plants such as anchoring, absorption of water and other essential nutrients (Shruthi, 2014). Our study also revealed that fresh and dry weight of the treated seedlings were significantly decreased in all the tested concentrations of the purified fraction. Similarly weight reduction was reported in several cases due to allelopathic action of different plants (Oyun, 2006 and Sahoo, 2010).

Seed vigor results from all properties which determine the rapid development of normal seedlings under various environmental conditions. (Baalbaki, 2009).The present study revealed a significant reduction in seed vigor index in all the tested concentrations of the purified fraction. Reduction in the Seed Vigor Index of several plants has been reported earlier due to allelopathic potential of different plants (Maisuria, 2009; Djanaguiramanet al., 2002)

IV. CONCLUSION

The present study further attests the significant allelopathic potential of methanolic leaf extract of Nyctanthes arbor – tristis (Antony S and P.J. Benny, 2017) by revealing the allelopathic effect of its purified fraction, MN F-10 on the germination and early growth of Vigna radiata seeds. Moreover, characterization of this fraction has led to the identification of the most possible compound as the allelochemical. However further detailed investigation is needed to confirm this finding including the chemical synthesis, activity determination and its comparison with the isolated compound. Although the current study has revealed the significant allelopathic potential of MN F-10 isolated from the methanolic leaf extract of the plant Nyctanthes

arbor- tristis, a detailed investigation is needed to explore its weedicidal effect as well as its various other broad-spectrum activities, that will make it as a perfect herbicide.

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