CHEMICAL STUDIES ON CONSTITUENTS OF SOME ANTIVIRAL PLANT EXTRACTS

By

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B.Sc. Agric. Sc. (Agricultural Biochemistry), Ain Shams University, 1997

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ABSTRACT

Mahmoud Shoukry Sadik El-Sayed: Chemical Studies on Constituents of Some Antiviral Plant Extracts. Unpublished Ph.D. Thesis, Department of Agricultural Biochemistry, Faculty of Agriculture, Ain Shams University, 2011

The biological activity (antiviral activity) of the extracts of black seeds (*Nigella sativa*) and neem leaves (*Azadirachta indica*) were determined against *Tobacco mosaic virus* (TMV) using *Nicotina glutinosa* and *Datura metel* as local lesion hosts. The general characters of this isolate was confirmed as it reacted with *Chenopodium quinoa* by appearing necrotic local lesion (NLL), forming amorphous inclusion bodies in the cytoplasm of TMV-infected tissue and electron microscopy of partial purified particles. The molecular confirmation by RT-PCR confirmed the presence of coat protein gene of TMV. The electron microscopy of virus preparation negatively stained with 2% uranyl acetate showed the presence of rod shaped TMV particles. At the level of Enzyme Linked Immuno Sorbent Assay (ELISA) detection, a compatible reaction between the virus (TMV) and its specific IgGs was found.

Study was conducted to evaluate the antiviral activity of extracts of neem leaves and black seeds. Data showed that neem leaves extract had antiviral activity higher than the black seed ones, and this could be due to its containing the sulfate group in the polysaccharide.

Data show that chemical composition of monosugars content for neem leaves using HPLC analysis were glucouronic acid, galactose, rhamnose and glucose were detected as relative concentration 32.56, 24.49, 22.45 and 20.41, respectively. The monosugars content of black seeds polysaccharide were xylose, glucose, mannose and rhamnose were detected as relative concentration 50.00, 32.14, 17.85, traces, respectively. IR spectra of polysaccharides isolated from neem leaves and Black seeds show the presence of many functional groups (OH, -CH_2- and non-symmetric and symmetric-CH_3) while IR spectrum of neem

polysaccharide revealed the presence of carboxyl and sulfate ester. However, the COOH group and sulfate ester were absent in the IR spectrum of black seeds. The SDS-PAGE analysis of purified protein from black seeds and neem leaves extract has shown 2 protein bands with a molecular weight from 17 to 55 KDa for black seeds and 4 protein bands from 20.066 to 89.123 KDa for neem leaves. The protein isolated from neem leaves and black seeds consist of (14) and (15) amino acids, respectively and the data indicated the presence of glutamic acid in the protein of black seeds while it is absent in the neem leaves.

The total phenolic and flavonoid compounds were 805.79 and 963.39 mg/100 g in neem leaves and 293.56 and 347.66 mg/100 g in black seeds, respectively and the highest amount of tannins and saponins 1438.87 and 9859.2 mg/100 g were found in neem leaves followed by black seeds 125.81 and 1267.2 mg/ 100 g, respectively. Thirteen phenolic and five flavonoid compounds were successfully identified using HPLC. The chemical composition (total carbohydrates, total proteins, total lipids and ash) of the two plants (Neem, Black seeds) also determined.

The genetic ISSR-PCR and RAPD-PCR analyses of genomic DNA of the four samples of tobacco plants (treated: TMV+Black seeds extract (1), TMV+Neem leaves extract (2) and Controls: TMV-infected (3) and TMV-free (4)) using a set of ten primers for each were used for amplification specific nucleotide fragments genomic DNA of such treated and control plant samples. The results showed that there is no effect of the tested extracts obtained from neem leaves or black seeds on DNA of the tobacco plants. At the same they inhibited the viral infection; this made them safe as biological substances could be used for biological control of the plant viruses.

Key Words: Neem, Black seeds, Plant extracts, antiviral constituents, polysaccharides, proteins, phenolic compound, flavonoid, HPLC, IR, SDS-PAGE, amino acid analyzer, *Tobacco mosaic virus* (TMV), ISSR-PCR and RAPD-PCR analyses.

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