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Cytotoxicity and Antioxidant Activity of Extractives from *Mirabilis jalapa*

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ABSTRACT

The petroleum ether, chloroform and methanol crude extracts of the two different plant parts (leaves and bark) of *Mirabilis jalapa* (Nyctaginaceae) were screened for cytotoxicity by brine shrimp lethality bioassay and the crude methanol extract of the bark was screened for antioxidant activity using the 1, 1-diphenyl-2-picrylhydrazyl-hydrate (DPPH) free radical scavenging assay. The petroleum ether extract of the bark showed significant cytotoxic activity with the LC_{50} value 8.12 µg/ml compared to vincristine sulphate (LC_{50} 0.33 µg/ml). On the other hand, the methanol crude extract of the bark showed mild antioxidant activity with the IC_{50} value 598.02 µg/ml compared to ascorbic acid(IC_{50} 70.985µg/ml). Above results suggest moderate cytotoxic and antioxidant activity of the extract.

Key Words: *Mirabilis jalapa*, Nyctaginaceae, Cytotoxicity, DPPH, Antioxidant.

INTRODUCTION

Plants produce a diverse range of bioactive molecules, making them a rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times (Farombi, 2003). Over 50% of all modern clinical drugs are of natural product origin (Stuffness et al., 1982) and natural products play an important role in drug development programs in the pharmaceutical industry (Baker et al., 1995). The relatively lower incidence of adverse reactions to plant preparations compared to modern conventional pharmaceuticals, coupled with their reduced cost, is encouraging both the consuming public and national health care institutions to consider plant medicines as alternatives to synthetic drugs (Nair et al., 2005). Thus plants are considered as are of the most important and interesting subjects that should be explored for the discovery and development of newer and safer drug candidates. *Mirabilis jalapa* (Bengali name – Krishnakali, Sandhayamalati; English name – 4 O'clock plant; Family- Nyctaginaceae) is a tall herbaceous climbing plant with opposite leaves, large showy flowers, coriaceous obovoid fruits and prominent tuberous roots, planted as an ornamental plant throughout the country (Ghani, 2003). Phytochemical investigations revealed that the plant contains alanine, alphaamyrins, arabinose, beta-amyrins, campesterol, daucosterol and dopamine (Yang et al., 2001). Previously, antimicrobial (Hamill et al., 2003), antinociceptive (Walker et al., 2008) and antigonorrhoeal (Caceres et al., 1995) properties of this plant have been reported. The present report demonstrates the cytotoxic and antioxidant properties of *M. jalapa* through *in vivo* and *in vitro* evaluation.

MATERIALS AND METHODS

Plant material

M. jalapa was collected from Siddeswari campus, Stamford University Bangladesh in January 2007 and identified by the laboratory of pharmacognosy and pharmacology, Stamford University, Dhaka, Bangladesh where a voucher specimen (SU-NNR-2008-15) for this collection has been retained. The leaves and the bark of the plant was first sun dried and then ground into coarse powder.

Extraction

The powdered leaf (250 g) of *M. jalapa* was separately extracted to exhaustion in a Soxhlet apparatus (50–70 °C) with petroleum ether, then chloroform and finally with methanol. All the extracts were filtered through a cotton plug followed by Whatman filter paper number 1 and then concentrated by using a rotary evaporator at low temperature (40-50 °C) and reduced pressure to provide petroleum ether (3.0 g), chloroform (3.5 g) and methanol (5.5 g) extractives of leaves. Similarly the powdered bark (250 g) of the plant was extracted by the same procedure to provide petroleum ether (4.26 g), chloroform (3.15 g) and methanol (4.85 g) extractives of bark.

Biological screening

Cytotoxicity study

Brine shrimp lethality bioassay (Persoone, 1980) technique was applied for the determination of cytotoxic property of petroleum ether, chloroform and methanol extractives of both the leaves and bark of the plant *M. jalapa*.

Preparation of positive control group

Vincristine sulphate was used as the positive control. Measured amount of the vincristine sulphate was dissolved in DMSO to get an initial concentration of 20 µg/ml from which serial dilutions are made using DMSO to get 10 µg/ml, 5 µg/ml, 2.5µg/ml, 1.25 µg/ml, 0.625 µg/ml, 0.3125 µg/ml, 0.15625 µg/ml, 0.078125 µg/ml and 0.0390 µg/ml. Then the positive control solutions are added to the premarked vials containing ten living brine shrimp nauplii in 5 ml simulated sea water to get the positive control groups

Preparation of negative control group

30 µl of DMSO was added to each of three premarked glass vials containing 5 ml of simulated seawater and 10 shrimp nauplii to use as control groups. If the brine shrimps in these vials show a rapid mortality rate, then the test is considered as invalid as the nauplii died due to some reason other than the cytotoxicity of the samples.

Counting of nauplii

After 24 hours, the vials were inspected using a magnifying glass and the number of survived nauplii in each vial were counted. From this data, the percent (%) of lethality of the brine shrimp nauplii was calculated for each concentration.

Screening for antioxidant activity

Antioxidant activity of the crude methanol extract of the bark of *M. jalapa* was determined on the basis of its scavenging potential of the stable DPPH free radical in both qualitative and quantitative assay.

i*) Qualitative assay:* A suitably diluted stock solutions were spotted on pre-coated silica gel TLC plates and the plates were developed in solvent systems of different polarities (polar, medium polar and non-polar) to resolve polar and non-polar components of the extracts. The plates were dried at room temperature and were sprayed with 0.02% DPPH in ethanol. Bleaching of DPPH by the resolved band was observed for 10 minutes and the color changes (yellow on purple background) were noted (Sadhu et al*.,* 2003).

ii) Quantitative assay: Quantitative assay was performed on the basis of the modified method of Gupta et al. 2003. Stock solution (10 mg/ml) of the plant extracts were prepared in ethanol from which serial dilutions were carried out to obtain concentrations of 1, 5, 10, 50, 100, 500 µg/ml. Diluted solutions (2 ml) were added to 2 ml of a 0.004% ethanol solutions of DPPH (Aldrich, USA), mixed and allowed to stand at 25 °C for 30 min for reaction to occur. The absorbance was determined as 517 nm and from these values corresponding percentage of inhibitions were calculated. Then % inhibitions were plotted against log concentration and from the graph IC_{50} was calculated. The experiment was performed in triplicate and average absorption was noted for each concentration. Ascorbic acid (Loba, India) was used as positive control.

RESULTS

Cytotoxicity study

Following the procedure of Mayer (Larson, 1988), the lethality of all the crude extracts to brine shrimp were determined on *A. salina.* From the bioassay, it was found that all the three crude

extracts (petrolium ether, chloroform and methanol extracts) of leaves and two crude extracts (chloroform & methanol extracts) of bark showed inactivity against brine shrimp nauplii. As a result, LC_{50} values could not be determined. For petroleum ether crude extract of the bark the LC_{50} value obtained from the best-fit line slope was found to be 8.12 µg/ml. In comparison with the positive control (vincristine sulphate, LC_{50} value 0.33 µg/ml), petroleum ether crude extract of the bark showed significant cytotoxic activity.

Antioxidant activity

DPPH is one of the free radicals widely used for testing preliminary radical scavenging activity of a compound or a plant extract.

i) Qualitative assay: The color changes (yellow on purple background) on the TLC plates were observed due to the bleaching of DPPH by the resolved bands.

ii) Quantitative assay: In the quantitative antioxidant study, it was found that IC_{50} value of the crude methanol extract of the bark of *M. jalapa* is more than 500 µg/mL which indicated the mild antioxidant activity of the plant. Table-1 shows the results of the antioxidant activity of the crude methanol extract and the standard, ascorbic acid.

DISCUSSION

Most of the polar compounds such as phenolic and flavonoid substances are potent inhibitors of reactive oxygen species attack (Owen et al., 2003). Phenolic and flavonoids also show cytotoxicity in Hoechst 33258 fluorescence assay by inhibiting cellular DNA in a concentration-dependent manner (Chang et al., 2003). The biological properties, including cytotoxic and antioxidant properties, of flavonoids are considered in an evaluation of the medicinal and nutritional values of these compounds (Harborne et al., 2000). From literature review and phytochemical analysis it was found that the plant, *M. jalapa,* contains phenolic as wll as flavonoid type compounds. Our present study results also reflected that crude methanol and petroleum ether extract of the bark showed mild antioxidant and significant cytotoxic activity respectively. In cytotoxicity study, other extracts showed inactivity against brine shrimp nauplii. The apparent cause for this variation among the various samples of the same species in the lethality effect may be attributed to concentrations of the active compound, due to variations in climatic conditions etc (Couladis et al., 2002).

Table 1: Evaluation of antioxidant activity of crude methanol extract of the bark of *M. jalapa*

Concentration (µg/ml)	Methanol extract of *M. jalapa* (Bark) % inhibition (mean ± SD)	% inhibition by Ascorbic acid (mean ± SD)
1	3.78 ± 0.0015	21.41 ± 0.0075
5	5.47 ± 0.009	42.76 ± 0.004
10	7.68 ± 0.008	56.98 ± 0.009
50	14.84 ± 0.0145	69.68 ± 0.004
100	23.05 ± 0.002	85.96 ± 0.011
500	40.68 ± 0.0045	94.17 ± 0.002
IC_{50} (µg/ml)	598.02	70.985

CONCLUSION

In the cytotoxicity study, the positive result of crude petroleum ether extract of bark suggests that the plant may contain antitumor or pesticidal compounds. The IC_{50} value of the crude methanol extract shows that *M. jalapa* is an important source of natural antioxidant also.

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