International Journal of Advanced Research in Botany (IJARB)
Volume 5, Issue 3, 2019, PP 21-26
ISSN No. (Online) 2455-4316
DOI: http://dx.doi.org/10.20431/2455-4316.0503004
www.arcjournals.org

Environmental impact of some poisonous weeds in Damietta, Egypt

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Abstract: *Toxic plant contains a number of toxic alkaloids produced as secondary metabolites, which are a characteristic feature of plants, are especially important and can protect plants against a wide variety of microorganisms. The antibacterial effect of aqueous and organic solvents (ethanol and acetone) extracts of four poisonous weeds (Datura stramonium, Chenopodium murales, Lotus corniculatus and Amaranthus viridis) obtained from Damietta province of Egypt, were tested in vitro against growth of bacteria, Escherichia coli and Helicobacter pylori.*

Alkaloid can use in antibacterial activity. Bacterial drug resistant was important problem against antibiotic so researcher going to used alkaloid extract as antibacterial activity. The effect of alkaloids of four plant have inhibitors of growth of bacteria (Escherichia coli and Helicobacter pylori). Results for alkaloid content in the plants (Datura stramonium (all plant), Datura stramonium (Seed), Chenopodium murales, Lotus corniculatus and Amaranthus viridis) have 150.5±6.1, 103.1±4.2, 30.7±2.4, 42.3±1.9 and 88.6±6.3 mg/g, respectively. The data of heavy metal concentration of soils within 0–20 Cm depth in different sites indicates that there is a variable changes in the concentration of the metals in the different sites.

The study confirms that all plants extracts used in this investigation possess in vitro antibacterial activity against the used organisms. The efficiency of the extracts varied with, solvent used in the extraction as well as, plant species and the part of plant used.

Keywords: *Toxic plant, alkaloids, antibacterial activity, antibiotic, heavy metals.*

1. Introduction

A wide variety of toxic plants produces compounds that may cause clinical symptoms in humans, some of which have caused severe poisonings. A few plants give rise to serious poisoning after ingestion even of a limited amount of plant material (Frohne et al., 2004). Alkaloid extracts of the leaves of plants known to have toxic effects when ingested by livestock were screened for biological activity (McGaw et al., 2005). Isolation of active compounds, in almost all cases, provided scientific validation for the use of the plants in traditional medicine. Although plants used medicinally are widely assumed to be safe, many are potentially toxic (Fennell et al., 2004). On the basis that toxic plants have proven pharmacological activity. Resistance in microbes against antibiotics is a worldwide problem that is caused because of frequent exposure of antibiotics. There are millions of chemical compounds that are being synthesized, thousands of which have been confirmed for their antimicrobial potential (Tim et al., 2014). Infectious diseases caused by these pathogenic microorganisms have become an important cause of death and mortality in immune-compromised patients in developing countries (Ara et al., 2009). Despite the availability of a wide range of antibiotics, bacteria are constantly developing resistance to these antibiotics (AL-Bari et al., 2006).

The present investigation aimed to study the effects of some extracts obtained from these four plants using different solvent (ethanol and acetone), on growth of some strains of *Escherichia coli* and *Helicobacter pylori*.

2. Materials and Methods

2.1. Study Area

Damietta Province is located in the downstream part of the Damietta branch of the River Nile at 31° 25' 10" north to 31° 48' 54" east N-32° 00' longitude to the north east of the Nile Delta region of Egypt.

The coast of Damietta Governate extends from El-Deeba village (about 20 kmfrom Port-said) to Gamasa at west along the Mediterranean Sea for about 42 Km. This province is bounded by Lake Manzala at the east, Mediterranean sea from the north and El-Dakahlia Governate from the west and the south. The total average area of Damietta Province is about 1029 Km^2 and the total agricultural area is about 115892 feddans (Mashaly, 2001).

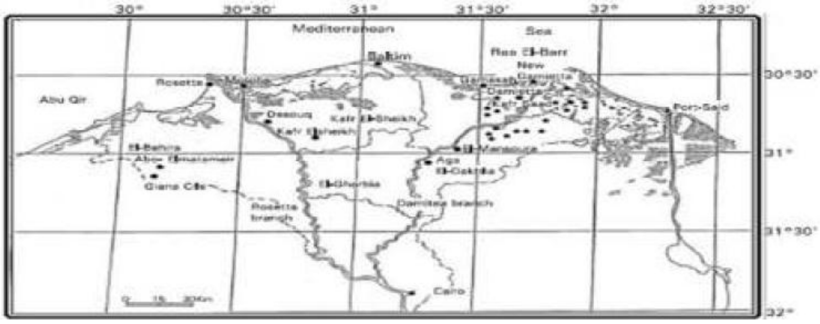

Fig1. *Location map of Damietta showing different sites where weeds and soil samples were collected.*

Four poisonous weeds (Datura stramonium, Chenopodium murales, Lotus corniculatus and Amaranthus viridis) were collected from 0.5 m × 0.5 m area during summer of 2017 across the Nile Delta coast from Damietta Province. After washing under running tap water, plants, were rinsed with distilled water and left to dry in the air under sunlight conditions. The air-dried plant material was oven-dried at 65 °C for 72 hours and then grinded to a fine powder prior to analysis.

2.2. Methodology

Presence of alkaloid was tested qualitatively by Dragondroff's method (Sreevidya and Mehrotra, 2003). Then subjected to quantitative estimation by UV-Spectrophotometer. This method is based on the reaction between alkaloid and bromocresol green (BCG).

2.3. Extraction

100 gm of each plant material was ground and then extracted with methanol for 24 hours in a continuous extraction (Soxhlet) apparatus. The extract was filtered and methanol was evaporated on a rotary evaporator under vacuum at a temperature of 45°C to dryness.

2.4. Qualitative Estimation (Test for Alkaloids)

Presence of alkaloid was confirmed by Dragendroff's method (Sreevidya and Mehrotra, 2003). A part of extract was dissolved in dilute HCL and 2 drops of Dragon drop's was added, a crystalline precipitate indicates presence of alkaloid. The sample which showed positive alkaloid was then subjected to further quantitative evaluation.

2.5. Separation of Alkaloid

A part of extract residue was dissolved in 2N HCl and then filtered. 1 ml of this solution was transferred to separatory funnel and washed with 10 ml chloroform (3 times). The pH of this solution was adjusted to neutral with 0.1 N NaOH. Then 5 ml of BCG solution and 5 ml of phosphate buffer were added to this solution. The mixture was shaken and complex extracted with 1, 2, 3 and 4 ml chloroform by vigorous shaking, the extract was then collected in a 10 ml volumetric flask and diluted with chloroform.

2.6. Preparation of Standard Curve

Accurately measured aliquots (0.4, 0.6, 0.8, 1 and 1.2 ml) of Atropine standard solution was transferred to different separatory funnels. Then 5 ml of pH 4.7 phosphate buffer and 5 ml of BCG solution was taken and the mixture was shaken with extract with 1, 2, 3, and 4 ml of chloroform. The extracts were then collected in 10 ml volumetric flask and then diluted to adjust solution with chloroform.

The absorbance of the complex in chloroform was measured at spectrum of 470 nm in UV-Spectrophotometer (SHIMADZU, UV-1800) against the blank prepared as above but without Atropine.

Spectrophotometric method is known for its simplicity, sensitivity, and rapid determination. This method is based on the reaction of alkaloid with bromocresol green, forming a yellow colored product (Trease and Evans, 2002; Shamsa et al., 2008).

2.7. Preparation of Bacterial Culture

The stock culture of E. coli 0157:H7 used was subcultured on MacConkey agar at 37 °C for 24 hrs. the culture was emulsified in 3 ml sterile saline and adjusted to obtain a different concentration. The stock culture of H. pylori strains. were isolated from antral mucosal biopsy specimens of patients at with chronic gastritis or duodenal ulcers The strains were identified on the basis of colony appearance, gram staining, and positive reactions in biochemical tests (catalase, urease, and oxidase). H. pylori strains were revived and cultured on brain heart infusion agar.

2.8. Preparation of Plant Extract

Antibacterial activities of different extracts from the four plant (Datura stramonium, Chenopodium murales, Lotus corniculatus and Amaranthus viridis) were used. The diluted extracts were prepared by using Dimethyl Sulfoxide (DMSO) to obtain the optimum dose concentrations of the different plant extract. DMSO was used as negative control. The antibacterial activity of each extracts were tested by Hole diffusion method (Al Somal et al., 1994). Muller Hinton agar plates were inoculated by rubbing sterile cotton swabs after immerse 100 µl bacterial suspensions on plates (overnight cultures grown at 37°C on nutrient agar and adjusted to 0.5 McFarland in sterile saline) over the entire surface of the plate. After inoculation, 9 mm diameter wells were cut into the surface of the agar using a sterile cork borer. The different dose concentrations were added to the wells . Plates were incubated at 37°C for 24 h. Control wells contained DMSO. Bacterial growth inhibition was determined as the diameter of the inhibition zones around the discs. Zones of inhibition were measured by using ruler. The diameter of zones wasrecorded. Each assay was carried out in triplicate.

2.9. Soil Sampling and Analysis

The sediments were collected from the bottom of the studied stations and were carried to the laboratory in a plastic bag. Shortly after the collection, the samples were spread over large glass plate; air dried, thoroughly mixed, and passed through 2 mm sieve to remove gravel and debris and then packed in plastic bags ready for analysis.

2.10. Methods of Digestion Soil

Digestion procedures were applied to digest soil, involving nitric acid-hydrogen peroxide, nitric acid-sulfuric acid, and nitric acid-perchloric acid mixtures. Add 0.05 g of soil and heated in hot plate, after completed digested completed to 20 ml by deionized H_2O.

2.11. Apparatus

Flame Atomic Absorption Spectrometer (Thermo Electron Corporation-S series) with deuterium lamp background Correction was used.

2.12. Determination of Heavy Metals in Soil

Final concentrations of the metals in the soil samples were calculated using the following formula (Uwah et al., 2012).

$$\text{Concentration (mg/kg)} = \frac{\text{Concentration} \quad (\text{mg/L}) \times V}{W}$$

Where V = Final volume (20 ml) of solution, and M = Initial weight (0.05 g) of sample measured.

2.13. Statistical Analysis

Data were analyzed using analysis of variance (SPSS, version 10.0, SPSS Inc., Chicago, Ill.). Means were separated with Duncan's multiple range tests (Kleinbaum et al., 1998 ; Moussa and Amira, 2017).

3. RESULTS AND DISCUSSION

3.1. Total Alkaloid Content

Alkaloid of toxic plants is directly linked to the wide range of chemical compounds synthesized by the various biochemical pathways. These compounds are classified as secondary plant products, because they are not much related to the plant's survival. The results for alkaloid content in the different plant Datura stramonium (all plant), Datura stramonium (Seed), Chenopodium murales, Lotus corniculatus and Amaranthus viridis have total alkaloid contents 150.5±6.1, 103.1±4.2, 30.7±2.4, 42.3±1.9 and 88.6±6.3 mg/g, respectively as showed in Figure (1). The content of alkaloid can use as antimicrobial (Tim et al., 2014; Kim et al., 2004). Results showed that: (i) screening of presence of alkaloid confirmed the presence of alkaloid in the plant organs (ii) The alkaloid contents of the plant organs of the plant species were significantly different from one another and (iii) The total alkaloid content of Datura stramonium was the highest among the four plant species.

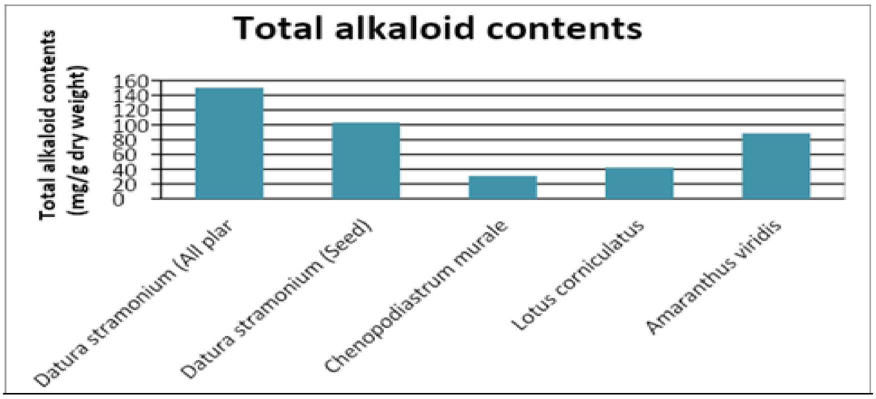

Fig2. *Total alkaloid contents from four plants,* Daturastramonium, Chenopodiummurales, Lotus corniculatus*and* Amaranthusviridis.

3.2. Antibacterial Activity of Alkaloid Extract of Toxic Plant

As shown in Tables (1, 2 and 3), the antibacterial activity showed positive response in all fractions of four plants extract (acetone and ethanol) as compared with antibiotic Amoxicillin, Clarithromycin and Trimethoprim. From this result we show that inhibition zone E. coli and H. pylori have good result can be improvement in future study. The antibacterial susceptibility tests showed that the alkaloid extract had potent antibacterial properties (Stavri et al., 2007; Tim et al., 2014).

Table1. *Antibacterial activity of Acetone extracted from four plants (Datura stramonium, Chenopodium murales, Lotus corniculatus and Amaranthus viridis*).

Plant	conc.%	Bacteria	Zone of inhibition (mm)
Daturastramonium (shoot, leaf and root)	**13**	*Escherichia coli*	8±0.05
	15	*Helicobacter pylori*	9±0.21
Daturastramonium (Seed)	**22**	*Escherichia coli*	7±0.11
	18	*Helicobacter pylori*	9±0.03
Chenopodium murales	**25**	*Escherichia coli*	8±0.02
	27	*Helicobacter pylori*	9+0.01
Lotus corniculatus	**26**	*Escherichia coli*	8±0.03
	24	*Helicobacter pylori*	12±0.09
Amaranthusviridis	**21**	*Escherichia coli*	9±0.07
	18	*Helicobacter pylori*	10±0.06

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values ± standard deviation. Mean inhibition zone diameter (mm) after 24 h of incubation.

Table2. *Antibacterial activity of Ethanol extracted from four plants* (*Daturastramonium, Chenopodium album, Lotus corniculatus*and *Amaranthusviridis).*

Plant	conc.%	Bacteria	Zone of inhibition (mm)
Daturastramonium(shoot, leaf and root)	15	*Escherichia coli*	7±0.07
	17	*Helicobacter pylori*	10±0.11
Daturastramonium(Seed)	20	*Escherichia coli*	5±0.09
	19	*Helicobacter pylori*	7±0.18
Chenopodium murales	23	*Escherichia coli*	6±0.22
	22	*Helicobacter pylori*	8±0.05
Lotus corniculatus	25	*Escherichia coli*	6±0.06
	22	*Helicobacter pylori*	11±0.11
Amaranthusviridis	20	*Escherichia coli*	8±0.07
	17	*Helicobacter pylori*	7±0.13

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values 1 standard deviation. Mean inhibition zone diameter (mm) after 24 h of incubation.

Table3. *Antibacterial activity of the antibiotic (Amoxicillin, Clarithromycin and Trimethoprim).*

Antibiotic	Bacteria	Zone of inhibition (mm)
Amoxicillin	*Escherichia coli*	**10±0.08**
	Helicobacter pylori	**13±0.05**
Clarithromycm	*Escherichia coli*	**8±0.11**
	Helicobacter pylori	**14±0.08**
Trimethoprim	*Escherichia coli*	**13±0.09**
	Helicobacter pylori	**8±0.21**

Each assay in these experiments was repeated three times and the results (mm of zone of inhibition) were expressed as average values ± standard deviation. Mean inhibition zone diameter (mm) after 24 h of incubation.

Table4. Plant species, life form, Life span, floristic category, and families associated with four plants (Datura stramonium, Chenopodium murales, Lotus corniculatus and Amaranthus viridis) in the Nile Delta coast.

Plant	Life form	Life span	Floristic category	Family
Avena fatua	Therophyte	Annuals	ME+MED	Poaceae
Imperatacylindrica	Graminoid	Perennials	NEO+	Poaceae
Chenopodiastrum murale	Therophyte	Annuals	COSM	Amaranthaceae
Tamarix nilotica	Phanerophyte	Perennials	ME+SA-AR	Tamaricaceae
Cynodon dactylon	Geophytes	Perennials	ME+IT	Aslepiadaceae
Juncus acutus	Cryptophytes	Perennials	ME+ES+IT ME+IR-TR+ER-ES+SU	Juncaceae
Malva parviflora	Therophyte	Annuals	ME+ER	Malvaceae
Rumex dentatus	Therophyte	Annuals	ME+IT+ES	Polygonaceae
Sonchus oleraceus	Therophyte	Annuals	COSM	Asteraceae
Cyperus rotundus	Geophytes	Perennials	PAN	Cyperaceae
Spergularia marina	Therophyte	biennial	ER-R+ME+IR-TR	Caryophyllaceae
Cynanchum acutum	Phanerophyte	Perennials	ME+IR-TR+ER-SR	Asclepiadaceae
Echinochloa stagnina	Therophyte	Perennials	PAL	Poaceae
Bassica indica	Therophyte	Annuals	ME+ER	Chenopodiaceae
Urospermum picroides	Therophyte	Annuals	ME+ER	Asteraceae
Beta vulgaris	Hemicryptophytes	Annuals	ME+ER-SR+IR-TR	Amaranthaceae
Urtica urens	Therophyte	Annuals	ME+ER	Urticaceae
Vicia sativa	Therophyte	Annuals	Me+IR+-IR+SA-AR	Fabaceae
Mesembryanthemum nodiflorum	Therophyte	Annuals	ME+ER	Aizoaceae
Solanum lycopersicum	Hemicryptophytes	Perennials	IR-TR+ER-SR+ME	Poaceae
Plantago major	Hemicryptophytes	Perennials	COSM	Plantaginaceae
Leptochloa fusca	Raunkiaer	Perennials	PAL	Poaceae
Solanum nigrum	Therophyte	Annuals	ME+ER-SR+IR-TR	Solanaceae
Rumex pectus	Therophyte	Annuals	ME+SA-AR	Polygonaceae
Ipomoea carnea jacq	Chamaephytes	Perennials	ME+IT+ES	Convolvulaceae

3.3. Life Form

The plants species were classified according to Raunkiaer (1937) classification into seven life form classes. There were represented in the present work as therophytes 56%, ge-helophytes 8%, chamaephytes 4%, hemicryptophytes 12%, cryptophytes 8%, and phanerophytes 12% (Table 4).

3.4. Plant Life Span

The 25-recorded can be classified based on life span into three groups: perennials 44%, annuals 52% and biennials 4%. Most of the recoded species are perennials and annuals with few biennials. These results are consistent with those of Boulos (1999 & 2005) as shown in (Table 4).

Table5. *Summary of heavy metal concentration of soils within 0–20 Cm depth in different sites.*

metal	site 1	site 2	site 3	site 4	site 5	site 6	site 7	site 8	site 9	site 0
Cu	16	9.6	20	51.6	27.6	27.2	12.4	13.6	12.4	12
Co	80	68.8	74.4	74	77.2	76.8	69.6	80	80.8	80.8
Ni	61.6	46.4	62.8	63.6	60.8	57.2	48	58	56.4	54.8
Zn	110.8	107.6	186.4	156.4	230	151.2	100	139.2	89.6	116
Mg	2664	2137.2	1231.6	848	1248.8	806	61.2	642.4	284.4	1159.6
Fe	351.6	214.8	686.4	732.4	772.8	654.8	297.6	462	256.4	116
Pb	184.8	179.6	176.4	190.4	196.8	185.6	216	218	229.6	220
Cd	23.6	22	19.2	24	26.8	24.4	23.6	28	29.2	26

The data of heavy metal concentration of soils within 0–20 Cm depth in different sites are shown in Table (5) which indicate that there is a variable chenges in the concentration of the metals in the different sites.

4. Conclusion

Use of toxic plant alkaloid extract for antimicrobial compounds was important of humanity is necessitated by the inherent ability of pathogens to develop resistance against antibiotics. Potentially harmful side effects associated with use of new chemical entities synthesized artificially and the unsustainably high costs of drug development are slowly shifting the focus to plant derived phytochemicals of medicinal significance (Scott et al., 2009).

Extracts of alkaloids from (*Datura stramonium*, *Chenopodium murales*, *Lotus corniculatus* and *Amaranthus viridis*) were found to be inhibitors of growth in bacteria (*Escherichia coli* and *Helicobacter pylori*), the standard antibiotic (Amoxicillin, Clarithromycin and Trimethoprim) used in that study to compared with extract of alkaloid (four plant).

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Citation: *Mohamed Adel Mohamed El-Gendey, et.al, "Environmental impact of some poisonous weeds in Damietta, Egypt". International Journal of Advanced Research in Botany (IJARB), vol. 5, no. 3, pp. 21-26, 2019. DOI: http://dx.doi.org/10.20431/2455-4316.0503004.*