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Analysis of alkaloid phytochemical compounds in the ethanolic extract of *Datura stramonium* and evaluation of antimicrobial activity

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The aim of this study was to assess the compounds of alkaloids extracts from the leaves of *Datura stramonium*, which can be the basis for the synthesis of new antibiotics. In this study, the alkaloid compounds of *D. stramonium* have been evaluated. The chemical compositions of the leaves of ethanolic extract of *D. stramonium* were investigated using gas chromatography-mass specroscopy. Gc-MS analysis of alkaloid leaves ethanolic extract of *D. stramonium* revealed the existence of the Ethyl iso-allocholate, D-asycarpidan-1-methanol, acetate (ester), 3-(1,5-dimethyl-hexyl)3a,10,10,12b-tetramethyl1,2,3,3a,4,6,8,9,10,10a,11,12,12a,12b-tetradec- ahydro -benzo[4,5] cyclohept,2,7-Diphenyl-1,6-dioxopyridazino [4,5:2,3] pyrrolo[4,5-d] pyridazine, 3,8,8-Trimethoxy-3-piperidyl-2,2-benaphthalene-1,1,4,4-tetrone, [5β] Pregnane3, 20β-diol,14α,18α-[4-methyl,3-oxo-[1-oxa-4-azabutane-1,4-diyl], diacetate, 1-monolinoleoylglycerol trimethylsilyl ether and 17-[1,5-dimethylhexyl]-10,13-dimethyl-3sstyrylhexadecahydrocyclopenta[a]phenathren-2-one. Alkaloids extract from leaves of *D. stramonium* were assayed for *in vitro* antibacterial activity against *Escherichia coli*, *Proteus mirabilis, Staphylococcus aureus, Pseudomonas aerogenosa* and *Klebsiella pneumonia* by using the diffusion method in agar. The zone of inhibition was compared with different standard antibiotics. The diameters of inhibition zones ranged from 0.8 to 2.01 mm for all treatments.

Key words: Alkaloids, antibacterial activity, *Datura stramonium*, gas chromatography-mass specroscopy.

INTRODUCTION

Datura stramonium is an annual herb, with stem erect and spreading branches above. It is common in the waste land, fields and gardens in Baghdad district (Figure 1). Leaves, seeds and roots contain the alkaloid daturine (a mixture of the two alkaloids hyoscyamine and atropine) and also contain scopolamine alkaloid (Hyosine) acids, tannin and fatty oil. Plants are rich source of secondary metabolites with interesting biological activities (Palombo and Semple, 2001; Koduru et al., 2006). Several plant products have been shown to exert a protective role

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Abbreviation: GC-MS, Gas chromatography-mass spectroscopy.

Figure 1. Leaves of *Datura stramonium*.

against the formation of free radicals and playing a beneficial role in maintaining disease condition (Ajitha and Rajanarayana, 2001). Very few of these chemicals are toxic also (Haraguchi et al., 1999; Sashikumar et al., 2003). The phytochemicals with adequate antibacterial activity will be used for the treatment of bacterial infections (Iwu et al., 1999; Purohit and Vyas, 2004; Krishnaraju et al., 2005). *Datura stramonium* is poisonous to cattle, horses, sheep, and children and causes the following symptoms: headache, nausea, vertigo, extreme thirsty, dry burning sensation in the skin, and in extreme cases death.

The main toxic alkaloids in *D. stramonium* are the tropane alkaloids which are the atropines (dl-hyoscyamine) and scopolamine (l-hyoscine) (Friedman, 2004; Steenkamp et al., 2004). Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants (Halpern, 2004). Intentional poisoning with *D. stramonium* has also been reported in several cases, namely a fatal poisoning with *D. stramonium* for its mind altering properties and the eating and chewing of Datura in a suicides attempt (Klein and Odera, 1984; Forrester, 2006; Monteriol et al., 2007). The toxicity of *D. stramonium* in grazing animals have been suspected by livestock owners and field veterinarians especially at time of drought or after ingesting freshly harvested maize that will be used for ensiling and heavily contaminated with young *D. stramonium*.

Successful extraction is largely dependent on the type of solvent used in the extraction procedure. The most often tested extracts are: water extract as a sample of extract that are primarily used in traditional medicine and extracts from organic solvents such as methanol, ethanol as well as ethyl acetate, acetone, chloroform, dichlormethane (Alves et al., 2000; Palombo and Semple, 2001; Uzun et al., 2004; Cos et al., 2006; Ncube et al., 2008; Stanojević et al., 2010). Considering the high economical and pharmacological importance of secondary plant metabolites, industries are deeply interested in utilizing plant tissue culture technique for large scale production of these substances.

MATERIALS AND METHODS

Collection and preparation of plant material

In this research, the leaves were dried at room temperature for ten days and when properly dried the leaves were powdered using clean pestle and mortar, and the powdered plant was size reduced with a sieve. The fine powder was then packed in airtight container to avoid the effect of humidity and then stored at room temperature (Imad et al., 2015).

Extraction and identification of alkaloids

The powdered leaves (2 g) were boiled in a water bath with 20 ml of 5% sulphuric acid in 50% ethanol. The mixture was cooled and filtered. A portion was reserved. Another portion of the filtrate was put in 100 ml of separating funnel and the solution was made alkaline by adding two drops of concentrated ammonia solution. Equal volume of chloroform was added and shaken gently to allow the layer to separate. The lower chloroform layer was run off into a second separating funnel. The ammoniacal layer was reserved. The chloroform layer was extracted with two quantities each of 5 ml of dilute sulphuric acid. The various extracts were then used for the following test:

Wagner's test

To the filtrate in test tube III, 1 ml of Wagner's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Dragendoff's test

To the filtrate in test tube II, 1 ml of Dragendoff's reagent was added drop by drop. Formation of a reddish-brown precipitate indicates the presence of alkaloids (Evans, 2002).

Mayer's test

To the filtrate in test tube I, 1 ml of mayer's reagent was added drop by drop. Formation of a greenish coloured or cream precipitate indicates the presence of alkaloids (Evans, 2002).

Table 1. Compounds present in the alkaloid extract of *Datura stramonium* using GC-MS analysis.

S/N	Alkaloid compound	Formula	Molecular Weight	Structure
1	Ethyl iso-allocholate	C26H44O5	436	Figure 2
2	D-asycarpidan-1-methanol, acetate (ester)	C20H26N2O2	326	Figure 3
3	3-(1,5-Dimethyl-hexyl) 3a,10,10,12b-tetramethyl 1,2,3,3a,4,6,8,9,10,10a,11,12,12a, 12b-tetradecahydro-benzo [4,5] cyclohept	C30H50	410	Figure 4
4	2,7-Diphenyl-1,6-dioxopyridazino[4,5:2,3] pyrrolo[4,5-d]pyridazine	C20H13N5O2	355	Figure 5
5	3,8,8-Trimethoxy-3-piperidyl-2,2-benaphthalene-1,1,4,4-tetrone	C28H25NO7	487	Figure 6
6	[5β]Pregnane3,20 β-diol,14α,18α-[4-methyl,3-oxo-[1-oxa-4-azabutane-1,4-diyl], diacetate	C28H43NO6	489	Figure 7
7	1-Monolinoleoylglycerol trimethylsilyl ether	C27H54O4Si2	498	Figure 8
8	17-[1,5-Dimethylhexyl]-10,13-dimethyl-3-sstyrylhexadecahydrocyclopenta[a]phenathren-2-one	C35H52O	488	Figure 9

Gas chromatography-mass spectroscopy (GC-MS) analysis

GC-MS analysis of the ethanol extract of *D. stramonium* was carried out using a Clarus 500 Perkin – elmer (Auto system XL) gas chromatograph equipped and coupled to a mass detector Turbo mass gold – Perkin Elmer Turbomass 5.1 spectrometer with an Elite – 1 (100% dimethyl poly siloxane), 30 m × 0.25 mm ID × 1 µm of capillary column. For GC-MS detection, an electron ionization system was operated in electron impact mode with ionization system operated in electron impact mode with ionization energy of 70 ev. The instrument was set to an initial temperature of 110°C, and maintained at this temperature for 2 min. At the end of this period, the oven temperature rose to 280°C, at the rate of an increase of 5°C/min, and maintained for 9 min. Helium gas (99.999%) was used as carrier gas at a constant flow rate of 1 ml/min, and an injection volume of 2 ml was employed (split ratio of 10:1). The injector temperature was maintained at 250°C, the ion-source temperature was 200°C, the oven temperature was programmed at 110°C (isothermal for 2 min), with an increase of 100°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 ev; a scan interval of 0.5 s and fragments from 45 to 450 Da. The solvent delay was 0 to 2 min and the total GC-MS running time was 36 min. The samples were injected in split mode as 10:1. Mass spectral scan range was set at 45 to 450 (m/z). The mass detector used in this analysis was Turbo-Mass Gold-Perkin Elmer and the software adopted to handle mass spectra and chromatograms was a Turbo-Mass ver 5.2 (Ameera et al., 2015; Huda et al., 2015).

Measurement of antibacterial activity

The antibacterial activity of alkaloids was determined by using agar well diffusion method. Wells of 5 mm diameter were punched in the agar medium with sterile cork borer and filled with plant alkaloid extract. Standard antibiotics, penicillin, kanamycin, cefotoxime, streptomycin and refampin (1 mg/ml) were also tested for their antibacterial activity. The plates were incubated at 370°C for 24 h. The negative control was added without adding the cultures to know the sterile conditions. The Petri dishes were placed in the refrigerator at 4°C or at room temperature for 1 h for diffusion, incubated at 37°C for 24 h, then the zone of inhibition produced by different antibiotics was observed. Measure it using a scale and record the average of two diameters of each zone of inhibition.

RESULTS AND DISCUSSION

GC-MS analysis of alkaloid compound clearly showed the presence of eight compounds. The alkaloid compound, formula, molecular weight and exact mass are presented in Table 1. Chromatogram GC-MS analysis of the ethanol extract of *D. stramonium* showed the presence of eight major peaks and the components corresponding to the peaks were determined as follows. The first set up peaks was determined to be ethyl iso-allocholate (Figure 2). The second peak was indicated to be D-asycarpidan-1-methanol, acetate (ester) (Figure 3). The next peaks was considered to be 3-(1,5-dimethyl-hexyl)3a,10,10,12b-tetramethyl1,2,3,3a4,6,8,9,10,10a,11,12,12a,12b-tetra decahydro-benzo[4,5]cyclohept, 2,7-diphenyl-1,6-dioxopyrid-azino[4,5:2,3] pyrrolo[4,5-d]pyridazine, 3,8,8-trimethoxy-3-piperidyl-2,2-benaphthalene-1,1,4,4-tetrone, [5β]Pregnane3,20 β-diol,14α,18α-[4-methyl,3-oxo-[1-oxa-4-azabutane-1,4-diyl], diacetate, 1-monolinoleoylglycerol trimethylsilyl ether, and 17-[1,5-dimethylhexyl]-10,13-dimethyl-3-sstyrylhexadecahydrocyclopenta [a]phena-thren -2-one (Figures 4 to 9). Among the identified phyto-compounds have the property of anti-oxidant and antimicrobial activities (Singh et al., 1998; Kumar et al., 2001; John and Senthilkumar, 2005; Venkatesan et al., 2005; Santh, 2006; Sazada et al., 2009). Plant based antimicrobials have enormous therapeutic potential as they can serve the purpose with lesser side effects.

Continued further exploration of plant derived

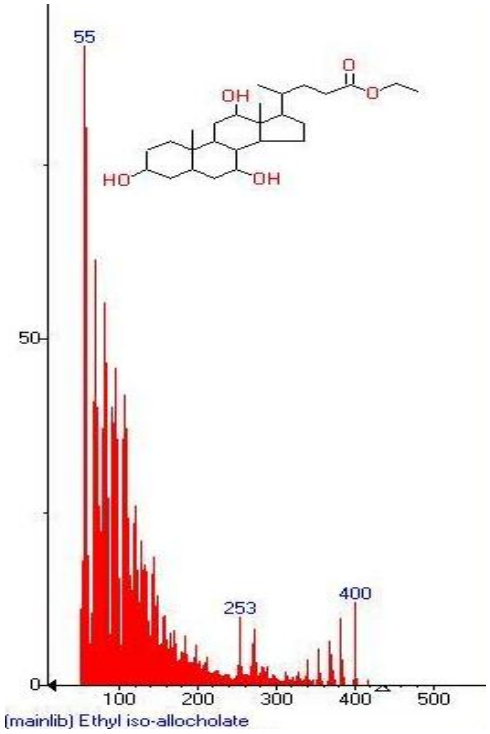

Figure 2. Structure of Ethyl iso-allocholate present in the leaves extract of *Datura stramonium* using GC-MS analysis.

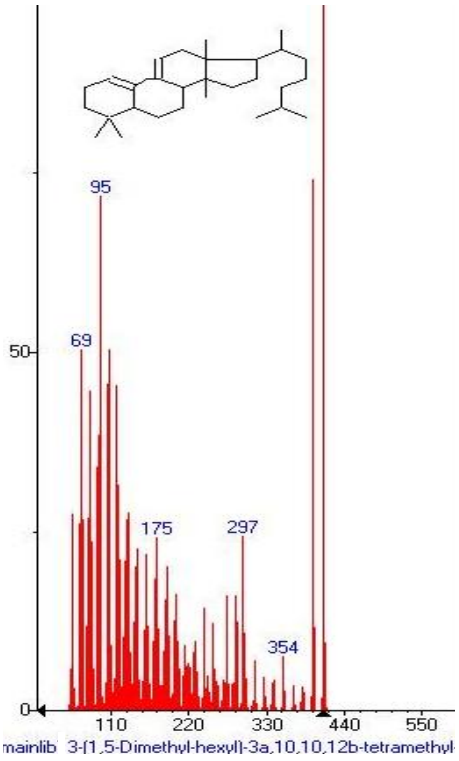

Figure 4. Structure of 3-(1,5-Dimethyl-hexyl)3a,10,10,12b tetramethyl 1, 2, 3, 3a, 4, 6, 8, 9, 10, 10a, 11, 12, 12a, 12b-tetradecahydro-benzo[4,5]cyclohept present in the leaves extract of *Datura stramonium* using GC-MS analysis.

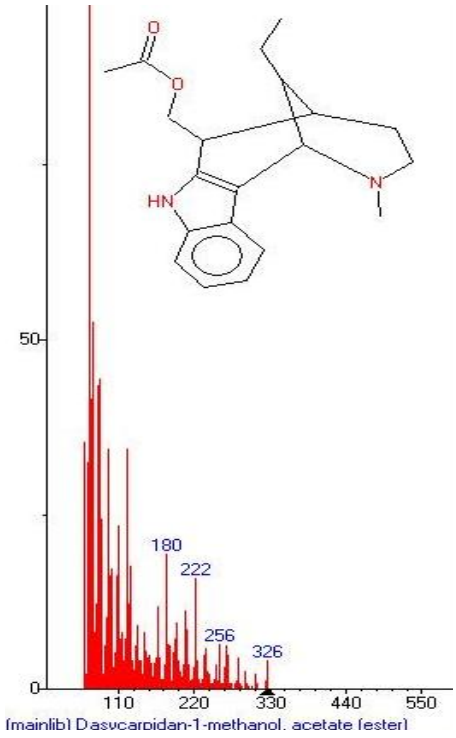

Figure 3. Structure of D-asycarpidan-1-methanol, acetate (ester) present in the leaves extract of *Datura stramonium* using GC-MS analysis.

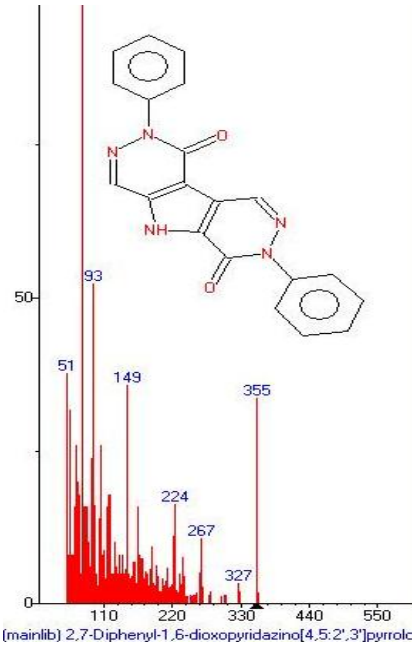

Figure 5. Structure of 2,7-Diphenyl-1,6-dioxopyridazino[4,5:2,3] pyrrolo[4,5-d]pyridazine present in the leaves extract of *Datura stramonium* using GC-MS analysis.

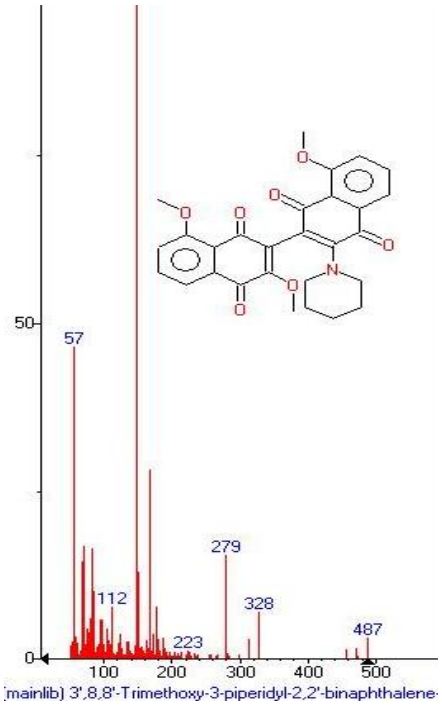

Figure 6. Structure of 3,8,8-trimethoxy-3-piperidyl-2,2-benaphthalene-1,1,4,4-tetrone present in the leaves extract of *Datura stramonium* using GC-MS analysis.

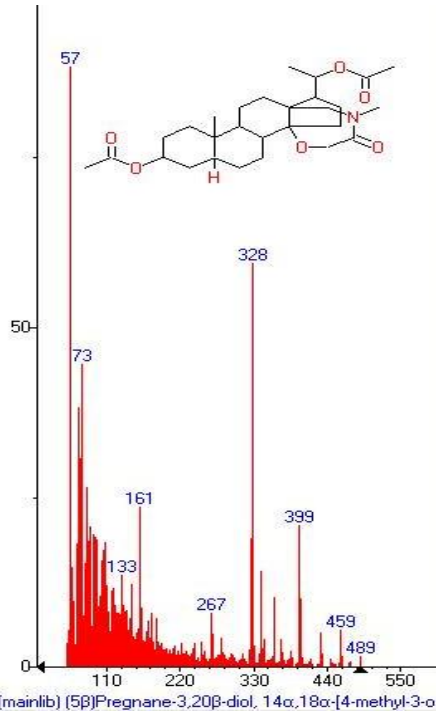

Figure 7. Structure of [5β]Pregnane3,20 β-diol,14α,18α-[4-methyl,3-oxo-[1-oxa-4-azabutane-1,4-diyl], diacetate present in the leaves extract of *Datura stramonium* using GC-MS analysis.

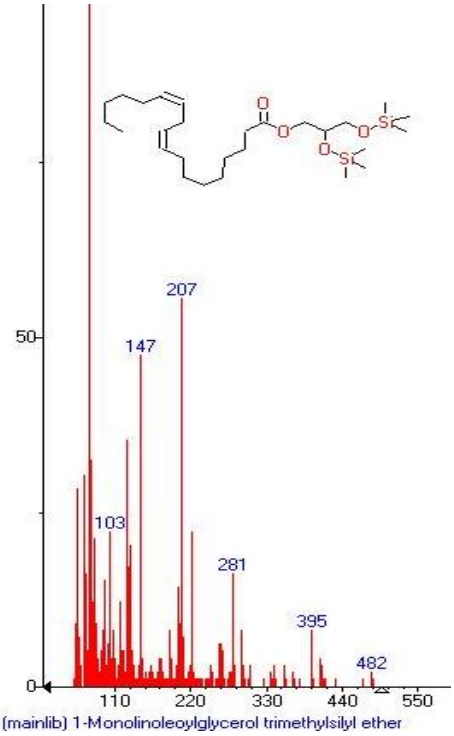

Figure 8. Structure of 1-monolinoleoylglycerol trimethylsilyl ether present in the leaves extract of *Datura stramonium* using GC-MS analysis.

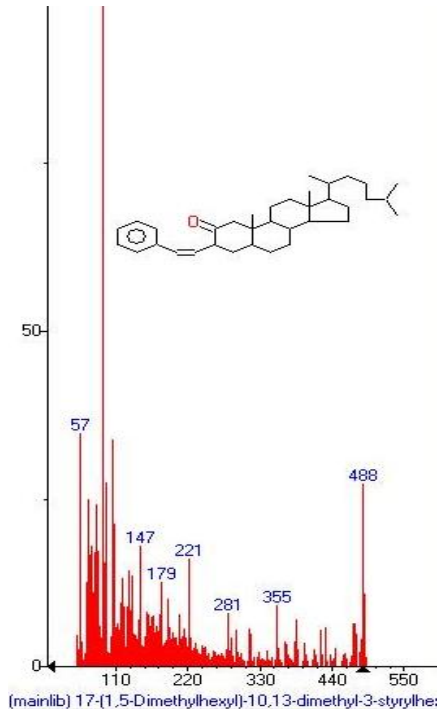

Figure 9. Structure of 17- [1,5-dimethylhexyl]-10,13-dimethyl-3-sstyrylhexadecahydro-cyclopenta[a]phenathren-2-one present in the leaves extract of *Datura stramonium* using GC-MS analysis.

Table 2. Zone of inhibition (mm) of test bacterial strains to alkaloid leaf extracts of *Datura stramonium* (L.) and standard antibiotics.

Alkaloid/Antibiotics	*Escherichia coli*	*Pseudomonas eurogenosa*	*Staphylococcus aureus*	*Proteus mirabilis*	*Klebsiella pneumonia*
Alkaloid	1.8±0.42	1.4±0.59	1.3±0.5	2.01±0.51	1.7±0.62
Kanamycin	0.9±0.1	0.5±0.4	0.6±0.2	0.4±0.1	0.8±0.3
Cefotoxime	1.1±0.3	1.5±0.1	1.2±0.1	1.2±0.3	1.3±0.5
Penicillin	1.6±0.1	1±0.5	1±0.4	1±0.2	1.1±0.2
Streptomycin	1.2±0.3	1.3±0.6	1.9±0.61	1.2±0.6	1.7±0.2
Rifampin	1.2±0.5	1.1±0.1	0.8±0.2	1.1±0.1	0.6±0.1

antimicrobials is needed today.

The results of the antimicrobial activity of extracts of leaves of *D. stramonium* are presented in Table 2. We observe that the sensitivity tests show the effect of crude extracted alkaloids from seeds and roots of different bacterial strains, giving varying diameters depending on the tested strains. The clear zone of growth inhibition was noted around the well due to diffusion of alkaloid compound. The diameter of the zone denotes the relative susceptibility of the test microorganism to a particular antimicrobial. The obtained results of the crude extracts were compared with the standard antibiotics such as penicillin, kanamycin, cefotoxime, Streptomycin and Refampin. All the tested organisms are highly sensitive to the ethanol leaf extract (1.4 to 2 mm) than the standard antibiotics which showed more or less activity (0.4 to 1.7 mm). The presence of antimicrobial substances in the higher plants is well established. Plants have provided a source of inspiration for novel drug compounds as plants derived medicines have made significant contribution towards human health. However, further studies are needed, including toxicity evaluation and purification of active antibacterial constituents from *D. stramonium* extracts looking toward a pharmaceutical use.

Conclusion

Eight chemical alkaloids constituents have been identified from ethanolic extract of the *D. stramonium* by gas chromatogram mass spectrometry (GC-MS). *In vitro* antibacterial evaluation of *D. stramonium* forms a primary platform for further phytochemical and pharmacological investigation for the development of new potential antimicrobial compounds.

Conflict of Interest

The authors did not declare any conflict of interest.

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