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Investigation of the Phytochemicals Present in the Local *Datura stramonium* Seeds by Using Two Extraction Methods

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Summary

In order to investigate the phytochemicals presence in the seeds of local plant of *Datura stramonium* rather than alkaloids. For this purpose two types of *Datura stramonium* extract were prepared. The first was precipitated by acetone and the other was destructed by ether then precipitated with ammonium sulphate. The first extract yielded 2.5gram/ 5 gram of seeds weight; while, the second extract yielded 1.23 gram/ 5 gram of seed weight. Biochemical tests exhibited that lectin and trace amounts of glycosides were presented in the acetone precipitated extract. Whereas, the ether extract contained lectin, tannin, flavonoids, and trace amounts of alkaloids.

الكشف عن ألمواد الكيمياوية الموجودة في بذور نبات الداتورة سترامونيوم المحلي بأستعمال طريقتي أستخلاص

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الخلاصة

لغرض الكشف عن المواد الكيمياوية غير القلويدات الموجودة في بذور نبات الداتورة المحلي. تم في هذا البحث اتباع طريقتين في تحضير مستخلصين من بذورهذا النبات. في الطريقة الاولى رسب المستخلص الاول باستعمال الاسيتون بينما استعمل الايثر في هرس البذور ومن ثم كبريتات الامونيوم في ترسيب المستخلص الثاني. اعطى المستخلص الاول 2,5غم لكل 5 غرامات من البذور في حين اعطى المستخلص الثاني 1,23غم لكل 5 غم من البذور. أظهرت الفحوص الكيمياوية وجود الاكتين وكميات قليلة من الكلايكوسيدات في المستخلص الاول بينما وجد في المستخلص الثاني كل من الاكتين و التانينات و الفلافينويدات بالاضافة الى كمية قليلة من القلويدات.

Introduction

Datura stamonium belongs to plantae kingdom, Magnoliophyta phyllum, Magnoliopsida class, solanales order and solanaceae family (1)

The solanaceae is a family of flowering plants, many of which are edible, while others are considered poisonous (2).

There are many different species in the *Datura* genus probably the most common types are *Datura stramonium, Datura innoxia, Datura metal, Datura ferox, Datura discolor,* and others (2). All of the species of *Datura* are leafy green plants pink to white flowers. The flowers are all fragrant, with *Datura innoxia* having a very distinct aroma, very hard to mistake with other plant. The seeds are found in small fruit which are completely covered with short, sharp, spines (hence the name thorn apple). The stalks are bristly and somewhat thin in comparison to the rest of the plant. The leaves are flat, mostly featureless and can either multi-edged (between 4 and 15 points) or ovoid (3; 4).

All parts of the thorn apple contain tropane alkaloids, the active compounds, but only the leaves and seeds have been recognized officially as drugs in pharmacopoeias (5) major of these alkaloids are hyoscyamin (generally the most abundant) and scopolamine, while atropine may be formed from hyoscyamine. Hyoscyamine is the predominant alkaloid in *D. stamonium* from the time of flowering on. Thorn apple leaves contain 0.2-0.45% of total alkaloids, seeds approximately 0.2% (1, 6).

The thorn apple alkaloid patterns of *Datura* are influenced more strongly by the environmental factors than genetic ones (7).

The aim of this study is to detect the compounds present in the seed of *D. stramonium* other than alkaloids.

Materials and Methods

Two methods of extraction were used in this study; the first was Precipitation by acetone and the second was extraction by ether.

Extraction of *Datura stramonium* Seeds by Acetone:

This method of extraction was carried out as follows (8):-

- Five grams of seeds were ground in a seed mill.
- The powder was extracted with 100 ml of PBS (prepared by dissolving 8.2 g Na_2HPO_4 and 8.82 g NaCl in 1000 ml distilled water and kept pH at 7.2) by stirring overnight at 4^{o}C. Then was filtrated and made up to 100 ml with phosphate buffer.
- The obtained extract was centrifuged at 30000 g for 1 hour.
- The supernatant incubated at 70^{o}C for 15 minutes and then cooled to 4^{o}C, before centrifugation at 10000 g for 15 minutes.
- The supernatant was cooled in an ice bath to 0^{o}C, followed by a slow addition of 23 ml of acetone at 0^{o}C and left for 10 minutes, before centrifugation at 10000 g for 10 minutes.
- After that, 161 ml of acetone was added to the supernatant and then centrifuged.
- The precipitate of the last centrifugation was dissolved in 75 ml buffer saline.
- The solution was dialyzed overnight against 5-liter phosphate buffer at 4 ^{o}C. Afterward, sample for biochemical test was taken while the remain was poured in Petri dish and dried at 37 ^{o}C.
- The dried extract was scrubbed off the Petri-dishes, placed in labeled, tightly sealed plastic tubes and stored in deep freeze at -20C°.

Extraction of *Datura stramonium* seeds by ether

This method was carried out according to (9):-

- In order to destruct the seeds, fifteen grams of seeds were ground 15-20 times with diethyl ether in a blender for 15 minutes of each time.
- The mixture was filtered by Whatman No.1 filterpaper.

- The resulting precipitate (powder) was extracted by stirring overnight at 4°C with 10 volume of cold PBS (prepared by dissolving 0.82 g Na_2HPO_4 and 11.76 g NaCl in 1000 ml distilled water and kept pH at 7.2).
- The solution was centrifuged in cooled centrifuged at 4000 rpm for 30 min.
- The volume of supernatant was estimated and fractionated with $(NH_4)_2SO_4$ ammonium sulfate at 0-40% [the determination of ammonium sulfate weight was occurred out according to the volume of supernatant and depending on the saturation table] at 4°C.
- The solution was centrifuged in a cold centrifuge at 4000 rpm for 30 minutes.
- The volume of supernatant was estimated and fractionated with ammonium sulfate at 40-60% at 4°C.
- After centrifugation by cooled centrifuge, the supernatant was fractionated with ammonium sulfate at 60-100%
- The obtained precipitate was re-dissolved in ordinary phosphate buffer and dialyzed against five litters of ordinary PBS at 4°C. Then sample for biochemical test was taken, while the remains poured off in the Petri-dishes for drying at 4°C.

The dried extract was scrubbed off the Petri-dishes, placed in labeled, tightly sealed plastic tube and stored in deep freeze at -20°C. Solutions of Biochemical Test Reagents

Test for Lectin

One drop of the extract was added to one drop of human blood on the slide. Agglutination should appear if lectin was present (10).

Test for Alkaloids

Marquis reagent: - Prepared by adding 1ml of formaldehyde to 10 ml of concentrated sulphuric acid H_2SO_4 (11).

Examinations for alkaloids, 2-3 drops of Marquis Indicator were added to 1ml of extract. If alkaloids were present, gray granules would appear.

Test for Tannin

Ferric chloride reagent: - This reagent was prepared by dissolving 1g of ferric chloride into 100 ml of distilled water.

Two- Three drops of prepared reagent were added to 1ml of sample. The presence of tannin is indicated by formation of blue color (12).

Test for Flavonoids

This test was carried out by adding two drops of concentrated H_2SO_4 to 1ml of the extract. If the Flavonoids were present, the color of solution should change to brownish red (11).

Test for Steroids

Two ml of chloroform were added to 1ml of extract then 2-3 drops of H_2SO_4 and 3 drops of acetic acid were added. The positive result is the change in color to brown and then after 10 minutes it will be changed to blue (11).

Test for Saponin

This test was carried out by:

a). Boiling 1-2 milliliters of the extract. If the Saponin was present, a foamy layer should appear (12).

b). Five ml of plant extract was added to (1-4 ml) of mercuric chloride (1% HgCl3). The presence of white precipitate was an indication of saponin (11).

Test for Glycosides

Three drops of H_2SO4 were added to the 1 ml of extract. Then 2-5 ml of Benedict's reagent were added and the mixture left in a boiling water bath for 5-10 minutes. If free sugars were present, a red precipitate should appear (11).

Results

The end product of *D. stramonium* seed by acetone precipitation was white yellowish powder. While the end product by ether extraction, was dark brown powder. The first extraction method yielded 2.5 gram from 5 grams weight of seeds but the second method yielded 3.7grams from 15 grams weight of seeds.

Table (1) shows the results of the chemical test for the general component of acetone and ether extracts of *D. stramonium.*

Table (1) Phytochemicals of the crude extracts of *D. stramonium*.

Phytochemicals	Acetone extract	Ether extract
Lectin	+	+
Tannins	_	+
Alkaloids	-	±
Flavonoids	-	+
Saponin	-	-
Steroids	-	-
Glycosides	±	

(-) means the extract does not contain the designated photochemicals
(+) means the extra contains the designated Phytochemicals.
(±) means the extract contains trace amount of designated Phytochemicals.

The acetone extract contained; lectin and trace amount of glycosides, but did not contain tannins, alkaloids, flavonoids, saponin, and steroids. While the ether extract contained; lectin, tannins, flavonoids, and trace amounts of alkaloids, but did not contain; saponin, steroid and glycosides.

Discussion

Datura stramonium was classified as medical herbal plant, had different medical properties. Seeds of this plant were used depending on that all phytochemicals (including lectin) of this plant were found in high concentration in the seeds (13). The first method for seed extraction was done by acetone. Heat stable proteins were precipitated by acetone which yielded crude lectin (*D. Stramonium* agglutinin) and other trace substance like glycosides. However, the second method which was performed by ether, heat labile and stable substances was precipitated by ammonium sulfate which yielded protein including crude lectin, and probably other types of protein, because the ammonium sulfate has ability to demonstrate lectin and enzymes (9). In addition, the chemical tests showed other phytochemicals such as; flavonoids, tannin and trace amount of alkaloids, in the second method of extraction.

Lectin was obtained in the first method by grounding seeds and extracted over night against high salt buffer. Whereas in the second method, the seeds were destroyed several times by ether then extracted over night against high salt buffer. Using high salt buffer was useful to dissolve most lectin present in the seeds. Research had demonstrated that the uncoated seed imbibing with high salt buffer eluted high proportion of lectin (14).

However, a study had demonstrated tannin and trace amounts of glycosides in *D. stramonium* extract (15). On the other hand flavonoids were reported by other studies (16, 17and 18). These studies found the type of flavonoids present in the *D. stramonium* which were coumarin and anthocyanin. In current study *D. Stramonium*

alkaloids were reduced by extraction with ether and removed by acetone method nearly completely.

References

1. Miraldi E Masti A Ferri, S and Coparini IB (2001). Distribution of hyoscyamine and scompolamin in *Datura stramonium*. Fitoterapia, 72(6):644-648.
2. Griffin W J and Lin GD (2000). Chemotoxonomy and geographical distribution of tropane alkaloids. Phytochemistry, 53(6):623-637.
3. Ellenhorn M Schwald S and Ordog G (1997). Ellenhorn's Medical Toxicology: diagnosis and treatment of human poisoning. 2nd ed. Baltimre (MD): Williams and Wilkins.
4. Spina SP and Taddei A (2007). Teenagers with Jimson weed (*Datura stramonium*) poisoning.
5. Grieve, M (1998). Modern herbal. Tiger Books International, London. pp: 804-805.
6. Dewick PM (1997). Medicinal natural products: a biosynthetic approach. John Wiley and Sons Chichester. Pp: 274-278.
7. Berkov S Zayed R and Doncheva T (2006). Alkaloid patterns in some varieties of *Datura stramonium*. Fitoterapia, 01,002.
8. Kilpatrick DC Yeoman MM (1978). Purification of the lectin from Datura stramonium. Biochem. J 175, 1151-1153.
9. Gasperi-Campani A Barbieri L Morelli P Stirpe F (1980). Seed extracts inhibiting protein synthesis in vitro. Biochem J 186, 439-441.
10. Crowley JF and Goldstein IJ (1982). Datura stramonium lectin. Method in Enzymology 83 Pp. 368-373.
11. Harborne JB(1973). Phytochemical methods, a guide to modern technique of plant analysis, Champan hall London. Pp 159-165.
12. Cannell P. (1998). How to approach the isolation of natural products 1st ed. Human press INC.
13. Kilpatrick DC Yeoman MM and Gould AR (1979). Tissue and subcellular distribution of the lectin from *Datura stramonium* (thron apple). Biochem J 184:215-219.
14. Broekaert WF Lambrechts D Verbelen J and Peumans WJ (1988). *Datura stramonium* agglutinin location in the seed and release upon immbibation. Plant physio. 86: 569-574.
15. Banso A and Adeyemo S (2006). Phytochemical screening and antimicrobial assessment of *Abutilon mauritianum, Bacopa monnifera* and *Datura stramonium*. Biokemistri 18(1):39-44.
16. Stone JL(2000). Does anthocyanin affect outcrossing rates in Datura stramonium (salanaceae)?. Amer J Botany. 87: 348-354.
17. Al-Kalid, MS (2005). Production of some alkaloids, from tow species of *Datura* plant, *Datura stramonium* and *D. innoxia* using plant tissue culture technique Ph D thesis Coll Sci Uni Bagh Iraq..
18. Moreira MD Piconco Mc and Barbasa LC (2007). Compound from ageratum conyzoides; isolation, structure elucidation and insecticidal activities. Pest Manaq Sci. 63(6):615-621.