Original Article

Toxicological Analysis of Synthetic Cannabinoids (Strox) Seized in Assiut Governorate Using Gas Chromatography/ Mass Spectrophotometry (GC/MS)

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ABSTRACT

Background: In recent years many unknown synthetic cannabinoids (SCs) and other components have appeared on the market as constituents of herbal mixtures known as "Strox" which is posing a major public health and legal risk for society. Additionally, there is no adequate data on the ingredients of the illegally available "Strox" and their pharmacological properties. **Aim:** This study was conducted to detect active principles of some SCs products and to investigate the diversity of available synthetic cannabinoids (Strox) in Assiut governorate in 2020. **Methodology:** Analysis of synthetic cannabinoid extract from three different seized samples (Strox) was conducted using gas chromatography/ mass spectrophotometry (GC/MS). **Results:** The gas chromatography/ mass spectrophotometry (GC/MS) analysis revealed that the different seized SCs samples were significantly different in terms of the active ingredients. Those ingredients included fatty acids, tobacco derivatives, cannabis sativa derivatives, benzodiazepines, quinazoline derivatives, piperine, indoles, analgesic agents, alkanes, melatonin derivatives, arsenic, solvents, and benzoic acid. **Conclusion:** The illegally available SCs samples have different ingredients which reflect the difference in the expected effects on users and making diagnosis of SCs use is challenging. Thus, leading to unpredictability of experienced symptoms and clinical presentation. **Recommendations:** Given the continuous emergence of variable mixtures containing new synthetic cannabinoids, a widespread cooperation system is necessary for sharing analytical information and improving drug market monitoring. This cooperation is mandatory to keep an up-to-date list of controlled substances.

KEYWORDS

Strox, New Psychoactive, Synthetic Cannabinoids, GC/MS, illegal herbal mixture.

I. INTRODUCTION

Synthetic cannabinoids (SCs) are new emerging human-made psychoactive chemicals which mimic the psychoactive effects of Δ9-tetrahydrocannabinol (Δ9-THC), the active ingredient in natural cannabis, by binding to the receptors CB_1 and CB_2(Vandrey et al., 2012). Over the past few years, SCs herbal mixture that invaded the drug abuse market in Egypt was called "Strox (El-Masry & Abdelkader, 2021). Many of them are structurally distinct from naturally occurring cannabinoids (Hudson & Ramsey, 2011). SCs are available in the form of dried plant blends. They are usually added to the plant material by soaking or spraying, using solvents such as acetone or ethanol to dissolve these substances, but in some cases their solid form (crystalline powder) is added to plant material (Alves et al., 2020). In most cases, the dried plants used in these mixes have no psychotropic effect, serving just as vehicles for SCs and providing the appearance of being of natural origin. However, some plants can contain alkaloids that are possibly psychoactive (Dresen et al., 2010, European Monitoring Centre for Drugs and Drug Addiction, 2017)

Cannabinoid contents and dosages vary substantially between lots, and even within the same package, according to analytical studies of psychotropic herbs. (Hillebrand et al., 2010). Manufacturers of SCs are well aware of the legal loopholes relating to chemical analogues, and they continue to alter SCs in order to maintain them lawful for distribution (Brewer & Collins, 2014). Therefore, new compounds are continually being developed, resulting in a never-ending supply of these products (Debruyne & Le Boisselier, 2015).

Several classifications have been proposed since the development of SCs, however some of them have been found to be inconclusive. The term "synthetic cannabinoids" refers to compounds with a variety of chemical structures that fall into one of the following conventional classifications (classical cannabinoids, non-classical cannabinoids, hybrid cannabinoids, eicosanoids, and others) (Hudson & Ramsey, 2011, Presley et al., 2013).

Many derivatives and analogues in the above-mentioned groups of compounds can be made by substituting one of the aromatic ring systems with a halogen, alkyl, alkoxy, or other substituent. Also, the length and arrangement of the alkyl chain can be changed without losing cannabinoid activity, and an indole can

be changed to an indazole, as well as a terminal fluorine replacement, permitting the synthesis of novel compounds (Diao & Huestis, 2017, ElSohly et al., 2019). Eventually, compounds with unanticipated pharmacological or toxicological effects derive from these structural alterations. (Gamage et al., 2018).

Multiple techniques for analyzing the presence of synthetic cannabinoids and/or their metabolites in human biological matrices, including blood, hair, plasma, and serum, have been tried to develop in recent years. These methodologies use a variety of analytical instruments, such as direct analysis in real time mass spectrometry (DART/MS) (Musah et al., 2012), liquid chromatography coupled with tandem mass spectrometry (LC/MS) (Teske et al., 2010), high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography-electrospray ionization tandem mass spectrometry (UPLC/MS), cheminformatics and immunoassays (Strano-Rossi et al., 2014, Ciolino, 2015).

Spice products have typically been studied using either GC/MS or LC/MS. The availability of pure reference materials, however, limits their identification and quantitation (Hermanns- Clausen et al., 2013, Bilici, 2014). The laboratories are unable to identify the unknown constituents quickly as these compounds may be not included in any mass or UV spectra library (Teske et al., 2010).

Despite increased concerns regarding the increased use of SCs All over Egypt in the past few years, there is no adequate data on the ingredients of the available illegal products 'Strox' and their pharmacological properties. The aim of the current study is to investigate the diversity of synthetic cannabinoids in Assiut governorate available in 2020.

II. MATERIAL AND METHOD: -

A- Chemicals

Synthetic cannabinoids: in the form of Strox samples obtained by official request Narcotics Bureau of Assiut Governorate (seized samples).

B- Instruments

Gas Chromatography/ Mass Spectrophotometer (GC/MS): Agilent GC-MS (7890A-5975B), column DB 5ms (30m*0.250mm*0.250µm). Analysis was done in Analytical Chemistry Laboratory (ACAL) at Faculty of Science - Assiut University.

Methods

1- Samples and their preparations:

Synthetic cannabinoid extract from three different Strox samples (obtained from Narcotics Bureau of Assiut Governorate seized samples) was prepared by crushing the buds/herbal matter in the sachets then dissolved in chloroform followed by ultra-sonication for 10 minutes and finally centrifuged for 5 minutes. The residue was subjected to GC/MS analysis.

2- GC/MS conditions:

Separation column, DB-5MS fused-silica capillary (30m* 0.25 mm* 0.25 µm film thickness; Agilent Technologies); max injector temperature, 280 °C; interface temperature, 150 °C; injection mode, splitless; injection volume, 2 µL; oven temperature setting, initial temperature at 40 °C for 2 minutes followed by ramping at 10 °C/min to 150 °C for 3 minutes, then ramping at 10 °C/min to 220 °C for 6 minutes, and then ramping at15°C/min to 280 °C for 15 minutes.

Helium carrier gas flow rate, 0.5mL/min; for 10.9 minutes, then 1 mL/min to1 mL/min; for 30 minutes. Run time 48 min with 2 min post run (rate 0.615 mL/min). This is the method of Simolka et al. (2012) modified by the Analytical Chemistry Laboratory (ACAL) at Faculty of Science - Assiut University.

3- The identification of the components:

Components were identified through the fragmentation pattern in the resulted mass spectra using mass spectral database expressed by retention time and peak (The time it takes for a solute to travel through a chromatography column is measured in retention time, which is computed as the time from injection until detection/ peak area reflects the amount of a specific analyte that's present)

III. RESULTS

Gas Chromatography Mass Spectrophotometry (GC/MS) analysis

Three different seized synthetic cannabinoids (Strox) samples were analyzed using GC/MS. The plant matrix was in the form of fluffy greenish material with some peculiar odor (most likely due to added flavors especially sample two with Vanillin odor) in the samples two and three. But the plant matrix of sample one was tobacco like with its characteristic odor.

The analysis of sample one using GC/MS demonstrated more than two hundreds compounds, the presenting are some of the founded ingredients: Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- (C10H14N2), Dihydromorphine , di(trimehylsilyl) ether,

3H-1,4-Benzodiazepin 2-amino-7-chloro-5-phenyl-, 4-oxide, Octadecanoic acid, 2-[(1-oxohexadecyl)oxy]ethyl ester), Oleic acid, eicosyl ester, Hexadecanoic acid, 2-(hexadecyloxy)ethyl ester, Dasycarpidan-1-methanol, 6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline, and many indole containing compounds.

On the other hand, the analysis of sample two demonstrated the presence of some indole containing compounds too but different from those in sample one, 2,3-Dihydro-6-methylfuro(2,3-b)quinolone, Quinoline N-oxide, Hexadecanoic acid methyl ester, Arsenosobenzene, and Vanillin.

Finally, the analysis of sample three revealed the presenting ingredients: 2,3,4,5-Tetrahydro-8,9-difluoroindolo[2,3-b] quinoxaline, 4-Morpholino-2-phenylquinazoline,5,N-Dimethyl-2[(1',2'dihydro5'methyl2'oxo3Hindol3'yliden e)hydrazineca, 7-Chloro-5-nitro-1H-indole, 5,6-indolediol, Ethyl 4-nitroindole-2-carboxylate, Hexadecanoic acid, Linoleic acid, 2-Ethoxy-2-thiophen-3-yl ethanol, and Piperine.

So, the analyzed products were completely different in terms of their ingredients although their appearance were the same and theall are so called Strox as a street name.

Tables (1,2,3) showed the ingredients of sample (one, two, and three respectively) analysis using GC/MS with demonstration of the retention time and peak area for each compound.

Table (1): Chemical compounds identified in Strox sample one by GC/MS

N	Name of the compound	RT (minutes)	PA (%)
1.	Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)-	20.55	16%
2.	4-tert-Butylphenoxy-.alpha.-propionic acid	12.627 min	15.196%.
3.	N-[2-[5-methoxy-1-(trimethylsilyl)-1H-indol-3-yl] ethyl]-N-(trimethyls) Acetamide (C19H32N2O2Si2)	35.81 min	13.579%.
4.	Neophytadiene	20.797 min.	8.077%.
5.	Benzoic acid	12.355 min.	7.194%.
6.	Octacosane	36.858 min	6.113%.
7.	Duvatriendiol	23.656 min.	4.409%
8.	1,5,9-trimethyl-12-(1-methylethyl)-4,8,13-Cyclot	24.141 min.	3.542%
9.	4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z)-	23.83 min.	2.662%.
10.	Heptacosane	32.873 min.	2.615%
11.	6,8-dibromo-2-(3-pyridyl)-4-phenyl-quinazoline	42.188 min.	2.433%
12.	3-{[N-(2'-Chloro-3'-pyridyl)aminocarbonyl]methyl}-indole	23.947 min	0.830%.
13.	Perhydro-2-butyl-spiro-5,2'-1',3'-dioxolane-Isoindole-1,3-dione	35.403 min.	0.322%.
14.	Hexadecanoic acid, 2-(hexadecyloxy)ethyl ester	51.42 min	0.31%
15.	Oleic acid, eicosyl ester	51.79 min	0.26%
16.	2,2',6,6'-Tetrabromo-3,3'-bi(1H-indole)	23.093 min.	0.187%
17.	Octadecanoic acid, 2-[(1-oxohexadecyl)oxy]ethyl ester),	51.54 min	0.09%
18.	3H-1,4-Benzodiazepin 2-amino-7-chloro-5-phenyl-, 4-oxide,	21.15 min	0.08%
19.	Dihydromorphine , di(trimehylsilyl) ether	50.89 min	0.06%
20.	Dasycarpidan-1-methanol, acetate (ester)	50.99 min	0.04%

- RT for retention time.
- PA for peak area.

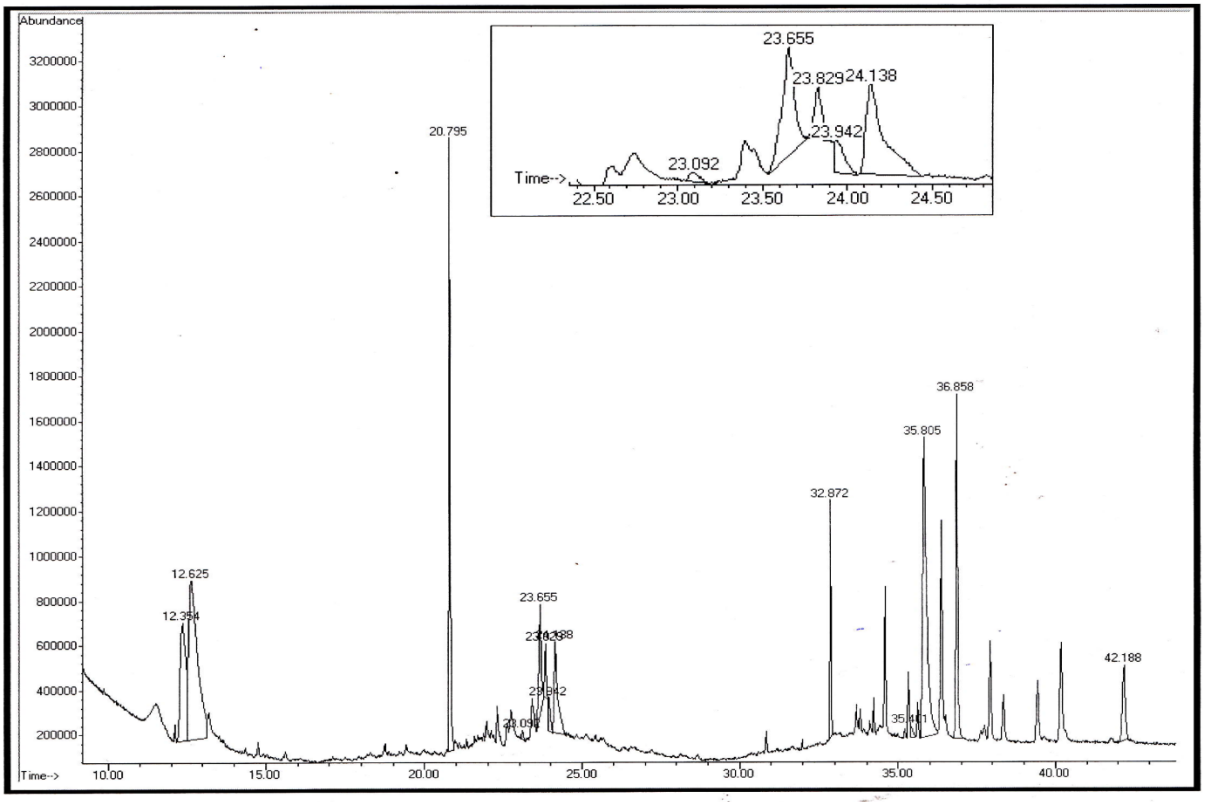

Figure (1): Chromatogram of sample one analysis using GC/MS.

Table (2): Chemical compounds identified in Strox sample two by GC/MS

N	Name of the compound	RT(minutes)	PA (%)
1.	Vanillin	14.748 min	60.578%.
2.	+-)-1-Ethoxy-3H-pyrrolo[1,2-a]indol-3-one	32.647 min	35.606%.
3.	2,4,5,5,8a-Pentamethyl-4a,5,6,7,8,8a-hexahydr o-2H-chromene	21.094 min	0.389%
4.	Arsenosobenzene	20.628 min	0.37%
5.	Quinoline N-oxide	32.369 min.	0.2%.
6.	Cis-Z-.alpha.-Bisabolene epoxide	20.02 min.	0.197%
7.	2,3-Dihydro-6-methylfuro(2,3-b)quinolone	31.612 min.	0.196%.
8.	Cineole	8.92 min.	0.177%.
9.	11-Octadecenoic acid, methyl ester	23.921 min.	0.176%
10.	n-Nonadecanoic acid	35.273 min	0.175%
11.	Hexadecanoic acid methyl ester	21.974 min.	0.164%
12.	1-Methyl-,1H-Indole-2,3-dione, 3-hydrazone	18.74 min	0.031%

- RT for retention time
- PA for peak area

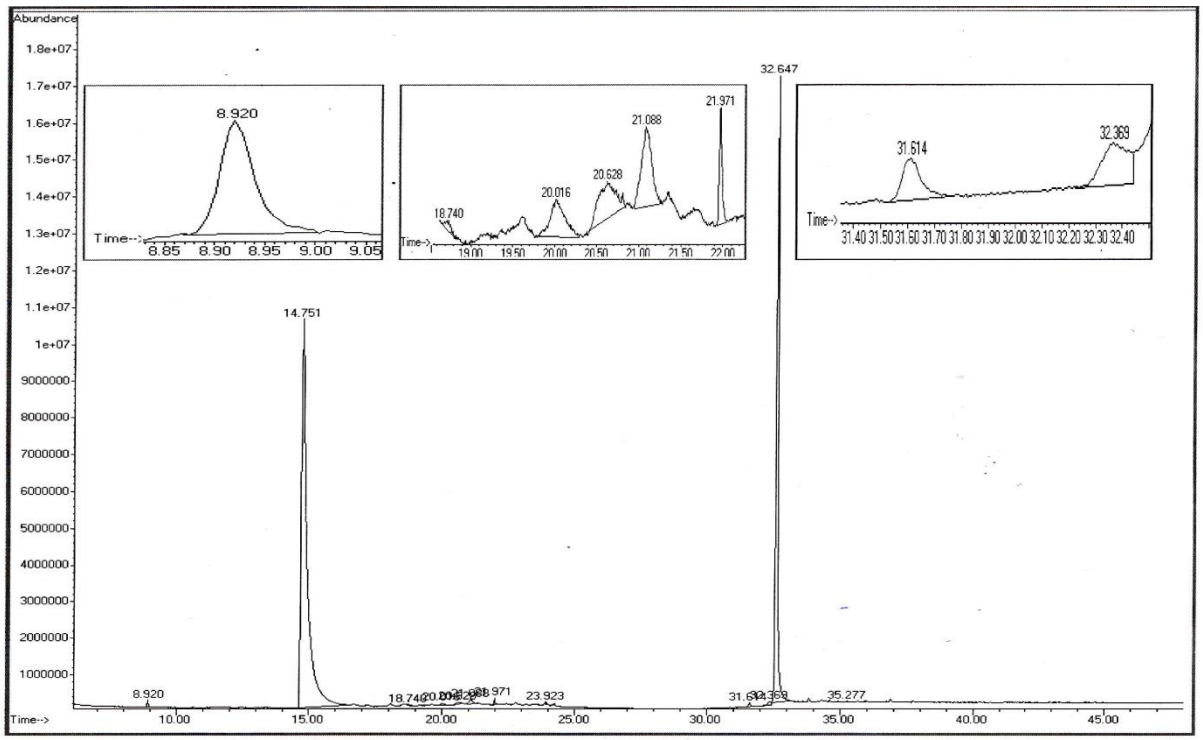

Figure (2): Chromatogram of sample two analysis using GC/MS.

Table (3): Chemical compounds identified in Strox sample three by GC/MS:-

N	Name of the compound	RT (minutes)	PA (%)
1.	Piperine	35.959 min	20.318%.
2.	3,3-dimethyl-Butanamide	13.262 min	14.259%
3.	4-Morpholino-2-phenyl quinazoline	26.638 min	8.013%.
4.	Hexadecanoic acid	22.802 min	5.483%.
5.	5(Z)-(1-(Allylpentylidene)-4-methoxy-4-methyl2-cyclopenten-1-one	23.339 min.	4.696%
6.	2-Ethoxy-2-thiophen-3-yl ethanol	11.87 min.	4.255%.
7.	Linoleic acid	24.936 min	3.937%
8.	Trans-octahydro-7a-methyl-1H-Inden-1-one	33.863 min.	3.013%.
9.	2,3,4,5-Tetrahydro-8,9-difluoroindolo[2,3-b] quinoxaline	23.746 min	1.009%.
10.	7-Chloro-5-nitro-1H-indole	25.739 min	0.595%.
11.	5, N-Dimethyl-2-[(1',2'-dihydro-5'-methyl-2'-oxo-3 H-indol-3'-ylidene)hydrazineca	23.85 min.	0.366%.
12.	5,6-indolediol	17.614 min	0.327%
13.	Ethyl 4-nitroindole-2-carboxylate	31.353 min	0.156%

- RT for retention time
- PA for peak area

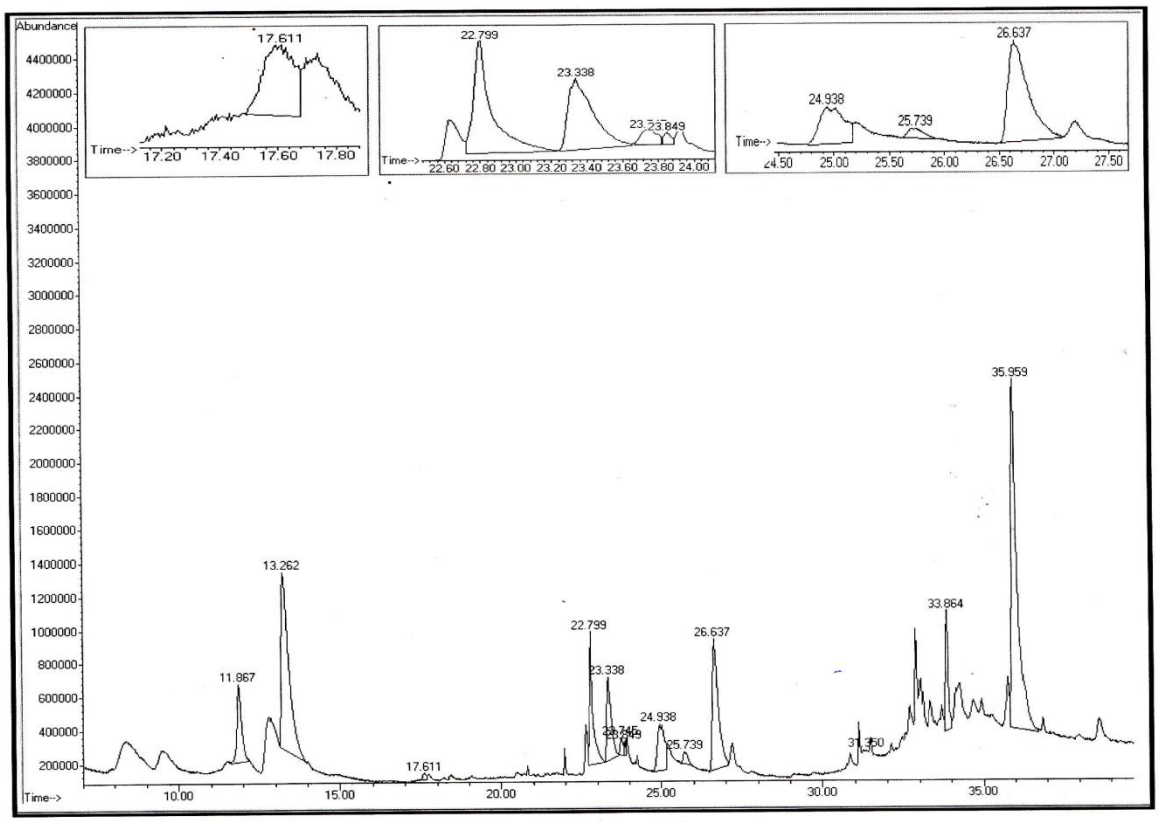

Figure (3): Chromatogram of sample three analysis using GC/MS.

IV. DISCUSSION

There is an increasing emergence of "Strox" users and there is a lack of knowledge about the components of these herbal mixtures in Assiut. So, the present study was conducted to detect the active principles of some SCs products used in Assiut governorate by Gas Chromatography/ Mass Spectrophotometry (GC/MS). (GC/MS) has some limitations mainly due to the low volatility of synthetic cannabinoids and resultant large variations. However, it is still useful as its maintenance procedures are simple and not so costly compared to those of LC/MS, and it is suitable for wide range identification and structural elucidation of synthetic cannabinoids (Choi et al., 2013).

The present results revealed that the three different seized SCs (Strox) samples using GC/MS were greatly different in terms of the active components. Those ingredients included fatty acids, tobacco derivatives, cannabis sativa derivatives, benzodiazepines, quinazoline derivative, Piperine, indoles,

analgesic agents, alkanes, melatonin derivatives, arsenic, solvents and benzoic acid.

The different compounds could be classified into plant and chemical origin. Plant origin substances that were detected in the specimens were nicotine Pyridine, 3-(1-methyl-2-pyrrolidinyl)-, (S)- (C10H14N2), Dihydromorphine, di(trimehylsilyl) ether, 3H-1,4-Benzodiazepin 2-amino-7-chloro-5-phenyl-, 4-oxide. In addition, Neophytadiene, a plant metabolite, detected from the inflorescences of cannabis sativa leaves by GC/MS (Ingallina et al., 2020). Quinazolines derivatives (heterocyclic natural alkaloids compound) were also present. They act as ligands for benzodiazepines and GABA receptors in the CNS and have some calcium channel blocking activity (Khan et al., 2015).

Fatty acids were also identified in samples; stearic acid (Octadecanoic acid, 2-[(1-oxohexadecyl) oxy]ethyl ester), Oleic acid, eicosyl ester, palmitic acid and (Hexadecanoic acid, 2-(hexadecyloxy)ethyl ester). These acids were among the most common fatty acids recognized in samples of cannabis (Piovesana et al., 2021). 4,7,10,13,16,19-Docosahexaenoic acid, methyl ester, (all-Z) is a fatty acid derivative, also detected in samples. Its presence is reported to be a potential interfering factor in gas chromatographic signals (Tettey et al.,2021). Male sex pheromone of Nezara viridula also detected.

Heptacasane and Octacosane are long chain alkanes, were previously found by GC/MS analysis of leaves of young marihuana plants (Cannabis sativa L.) and hashish samples. It was found that these alkanes can interfere with the qualitative and quantitative detection of cannabinoid by GC/MS as they have retention times similar to those of the major cannabinoids (De Zeeuw et al., 1973).

A potential fluorinated quinoline CB2 agonist is detected in sample (2); 2,3-Dihydro-6-methylfuro(2,3-b)quinolone. 2,3,4,5-Tetrahydro-8,9-difluoroindolo[2,3-b] quinoxaline was detected in sample (3). Saari et al. (2011) have reported that the derivatives of quinoxaline had a low-potency partial CB2 receptor agonist action. 7-Chloro-5-nitro-1H-indole (7-Chloro-5-nitroindole) and Ethyl 4-nitroindole-2-carboxylate were detected in sample (3). 5-Nitroindole I is a Cannabinoid receptor type 1 (CB1) antagonist, however many substituted indoles have been recognized as clandestine synthetic cannabinoids.

Furthermore, multiple chemicals are mixed during the manufacturing and releasing of these substances into the market is another challenging factor in the interpretation (Gurdal et al., 2013). For instance, (El-Masry & Abdelkader, 2021) results of analyzing stox packages revealed the presence of xylene, methylenedioxymethamphetamine (MDMA) , and trihexyphenidyl with its anticholinergic effect (in some packages) among other unidentified substances. On the other hand, the analysis of multiple samples of "voodoo" another street name of used synthetic cannabinoid in Egypt showed that samples shared the psychoactive compounds THC, amphetamine, MDA, oxazepam, and tramadol. Some of them also contained methadone, and another contained diazepam instead of methadone (Hussien et al., 2022). The analyzed samples in the present study also share the presence of cannabis derivatives and benzodiazepines and morphine derivatives but differ in the others. Additionally, both studies reported the presence of several unknown compounds. So, there is a wide diversity of these compounds among different street names of synthetic cannabinoid and in the same street name used also.

V. CONCLUSION

The illegally available SCs "Strox" samples have different ingredients which reflects the difference in the expected effects on users and making diagnosis of SCs using is challenging. Thus, leading to unpredictability of experienced symptoms and clinical presentation.

VI. LIMITATIONS OF THE STUDY

Although some synthetic cannabinoids can be detected by chromatography, the utility of these tests is limited by the availability of the instrument, understanding of the method, time consuming, and cost.

GC/MS is not capable of directly analyzing drugs that are nonvolatile, polar, or thermally labile. Identification of some of the unknown components is difficult because currently these compounds are not included in any mass or UV spectra library.

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التحليل السمي لأشباه القنب الاصطناعية (ستروكس) المضبوطة في محافظة أسيوط باستخدام كروماتوغرافيا الغاز / قياس الطيف الكتلي(GC/MS)

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في السنوات الأخيرة ، ظهرت العديد من اشباه القنب الاصطناعي ومركبات اخري في السوق كمكونات لخلطات عشبية تُعرف باسم "ستروكس" والتي تشكل خطرًا كبيرًا على الصحة العامة ومخاطر قانونية على المجتمع. بالإضافة إلى ذلك ، لا توجد بيانات كافية عن مكونات "ستروكس" المتاحة بشكل غير قانوني وخصائصها الدوائية. **الهدف من الدراسة:**أجريت هذه الدراسة للكشف عن المكونات النشطه لبعض اشباه القنب الاصطناعي والتحقق من تنوع اشباه القنت الاصطناعي "ستروكس" المتداول في محافظة أسيوط في عام 2020. الدراسه عباره عن تحليل لمستخلص القنب الصناعي "ستروكس" من 3 عينات مختلفة مضبوطة بإستخدام كروماتوغرافيا الغاز- مطياف الكتلة. **النتائج:** كشف تحليل كروماتوغرافيا الغاز / مطياف الكتله (GC / MS) أن عينات اشباه القنب الاصطناعية المضبوطة الثلاثه كانت مختلفة بشكل كبير من حيث المكونات النشطة. تضمنت هذه المكونات الأحماض الدهنية ومشتقات التبغ ومشتقات القنب ساتيفا والبيبيرين والإندول والعوامل المسكنة والألكانات ومشتقات الميلاتونين والزرنيخ والمذيبات وحمض البنزويك. **الخلاصة:** تحتوي عينات القنب المخلق المتاحه بشكل غير قانوني"ستروكس" علي مكونات مختلفه تعكس الاختلاف في التأثيرات المتوقعة علي المستخدمين وتجعل تشخيص استخدامها امرا صعبا. مما يؤدي إلى عدم القدرة على التنبؤ بالأعراض السريريه من ذوي الخبرة. **التوصيات:** نظرًا للظهور المستمر للمخاليط المحتوية على اشباه القنّب الاصطناعية الجديدة، اصبح من الضروري وجود نظام تعاون واسع النطاق لتبادل المعلومات التحليلية وتحسين مراقبة سوق الأدوية. وهذا التعاون أمر ضروري لتحديث قائمة المواد الخاضعة للرقابة.