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Secondary Metabolites from Plants: The Thin Border Between Beneficent and Harmful

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ABSTRACT: The main family of phytochemical compounds derived from naturally synthesised secondary metabolites are alkaloids, terpenoids and phenolics. Recently, these compounds known also as novel chemical entities (NCE) have been used as drug precursors or templates for synthetic moieties. Also, their potential for pharmacological applications has been as well extensively investigated. However, in recent years, a serious problem worldwide has become the use of illegal drugs, where to potential of some secondary metabolites to act as bio- or a psychoactive component is often refined. Considering that, forensic analysis of secondary metabolites from plants is of the utmost importance. Moreover, great effort has been given to developing testing strategies capable of identifying and quantifying secondary metabolites from various precursors over the past few years. Chromatography is a powerful instrumental technique in the analyses of selected NCE and seems entirely to fulfil the requirements of various laboratories all over the world.

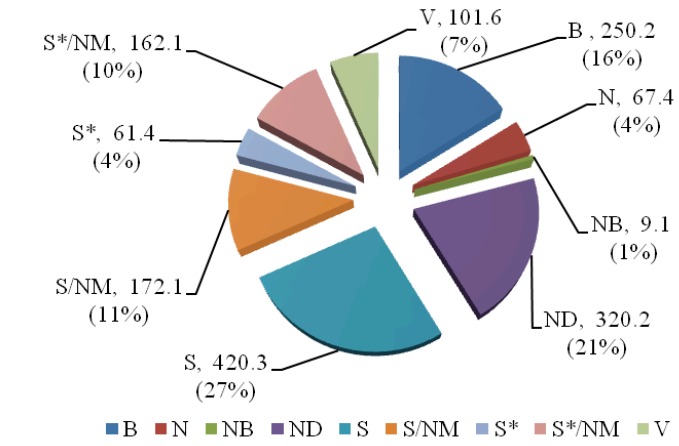
KEYWORDS: chromatography, drug precursors, entities (NCE), forensic analysis, novel chemical, secondary metabolites

Introduction

The world of plants, animals and micro-organisms represent a “reservoir of natural compounds” that offer a variety of species used today as remedies for many diseases and also provide a complex of organic chemical combinations in many parts of the world (Brusotti, Cesari, Dentamaro, Caccialanza and Massolini 2014, 218; Malutan and Popa 2007, 1).

Natural products and their derivatives used as sources for extracts or pure compounds have been a valuable source of therapeutic agents throughout history. Moreover, higher plants, as sources of medicinal compounds, have continued to play a dominant role in maintaining human health since antiquity. It is reported that over 50% of all medicines on the market are of natural origin and these natural products play an important role in drug development programs in the pharmaceutical industry (Kiruthika and Sornaraj 2011, 2025). However, the biological activity of plant species may remain largely unexplored (Brusotti, Cesari, Dentamaro, Caccialanza and Massolini 2014, 218).

Newman and Cragg (2016) report in a recent study that "natural product and/or natural product structures continue to play a highly significant role in the drug discovery and development process", so biodiversity is an unlimited source of new chemicals entities, NCE), leading to the emergence of potential new drugs on the market. Also, they report 1564 new approved drugs between 1981-2014 (Figure 1). These new chemical entities (NCEs) are secondary metabolites synthesized from plants and can be classified into three main chemical groups: alkaloids, terpenoids and phenolics (Newman and Cragg 2016, 630-631, Brusotti, Cesari, Dentamaro, Caccialanza and Massolini 2014, 218).



B - Biological macromolecule
N - Unaltered natural product
NB - Botanical drug
ND - Natural product derivative
NM - Mimic of natural product
S - Synthetic drug (from random screening and modification of existing agent)
S* - Synthetic drug (pharmacophore from natural products)
V - Vaccine

Figure 1. Drug Discovery from Natural Products as New Chemical Entities with 1564 New Approved Drugs or 100% (1981 – 2014)

Secondary herbal metabolites present chemical diversity, including many compounds, such as vitamins, nutrients, antioxidants, anti-carcinogens as antimicrobial, antifungal and antibacterial agents with an important role in medicine. Plants with a high content of antioxidants such as vitamins and phenolics are considered to be effective in preventing these diseases by reducing oxidative stress and blocking the chemical process of lipid peroxidation in biological systems. Recent studies have also indicated that the therapeutic benefits of certain raw herbal medicines have been derived from their antioxidant activities. Lately, scientists and researchers have turned their attention to raw extracts and pure active compounds isolated from plant species used in various remedies. Now it is essential to isolate, identify and characterize the new phytochemical compounds of locally grown medicinal plants commonly used by phytotherapists to treat various diseases (Razia and Sivaramakrishnan 2014, 756; Rates 2001, 603; Hossain, ALsabari, Weli and Al-Riyami 2013, 209).

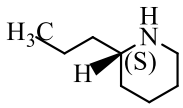
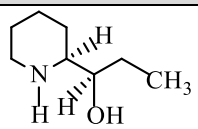
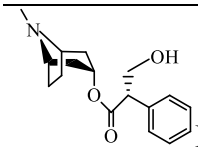
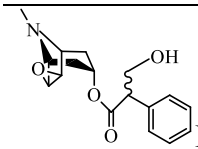
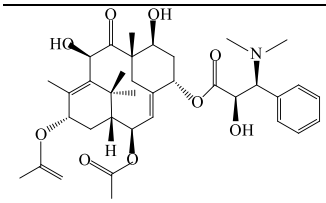
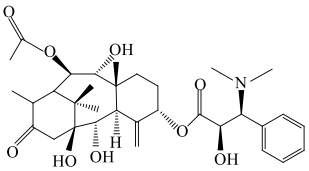
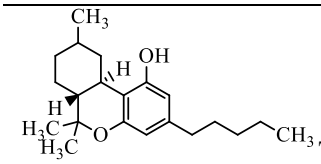
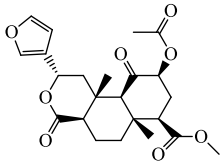
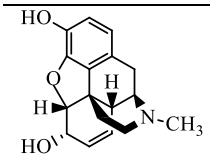
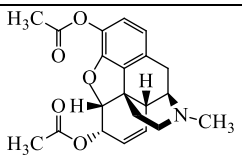
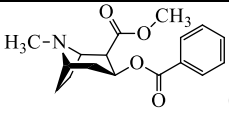
Ethnobotanical plants and associated bioactive compounds

In the past, the use of hallucinogenic substances has been associated with magic and ritual. These substances were also used as recreational drugs and consequently could lead to accommodation problems. The major plants containing hallucinogenic or psychoactive substances used in the natural form are: *Conium maculatum* (coniine and conhydrine), *Atropa belladonna,*(hyoscyamine), *Hyoscyamus Niger* (hyoscine), *Taxus baccata (Taxine A and Taxine B), Cannabis sativa* (tetrahydrocannabinol), *Salvia divinorum* (Salvinorim A), *Papaver somniferum* (morphine and heroine), *Erythroxylum coca* (cocaine) (Table 1).

A number of these plants have found applications in modern medicine, either in natural form or as coordinating compounds for optimizing processes in organic synthesis chemistry. Moreover, it is known that differences in the chemical composition of the above-mentioned plant varieties may produce different effects on humans (Salim, Chin and Kinghorn 2008, 2).

For example, there are synthetic cannabinoids that were originally developed as a potential source of analgesic drugs: **Dronabinol** [(−)-*trans*-Δ^9-tetrahydrocannabinol] sold as a drug under the name Marinol (Drug Bank n.d., Dronabinol), **Nabilone** [(6a*R*,10a*R*)-*rel*-1-hydroxy-6,6-dimethyl-3-(2-methyloctan-2-yl)-], Analgesic. Used for therapeutic purposes, chemotherapy, anorexia, treatment of AIDS patients, multiple sclerosis and the chemical composition is similar to cannabis (Drug Bank n.d., Nabilone), **Sativex (Nabiximols)**, Analgesic. It's a certified cannabinoid, used as a spray for neuropathic pain, spasms, severe bleeding. Currently, studies are being done to be used in the treatment of stage III cancer patients (Drug Bank n.d., Nabiximols).

Table 1. Plants and Specific Chemical Compounds Between Beneficent and Harmful

Compound	Scientific clasification	
 Coniine  Conhydrine	***Conium maculatum*** Order: Apiales Family: Apiaceae Genus: *Conium* Species: *C. maculatum*	
 Hyoscyamine	***Atropa belladonna*** Order: Solanales Family: Solanaceae Genus: *Atropa* Species: *A. belladonna*	
 Hyoscine	***Hyoscyamus Niger*** Order: Solanales Family: Solanaceae Genus: *Hyoscyamus* Species: *H. niger*	
 Taxine A  Taxine B	***Taxus baccata*** Order: Pinales Family: Taxaceae Genus: *Taxus* Species: *T. baccata*	
 Tetrahydrocannabinol	***Cannabis sativa*** Order: Rosales Family: Cannabaceae Genus: *Cannabis* Species: *C. sativa*	
 Salvinorin A	***Salvia divinorum*** Order: Lamiales Family: Lamiaceae Genus: *Salvia* Species: *S. divinorum*	
 Morphine  Heroine	***Papaver somniferum*** Order: Ranunculales Family: Papaveraceae Genus: *Papaver* Species: *P. somniferum*	
 Cocaine	***Erythroxylum coca*** Order: Malpighiales Family: Erythroxylaceae Genus: *Erythroxylum* Species: *E. coca*	

Coniine alkaloid has been used as a sedative and for its antispasmodic properties (Binev 2013, 903). Conium is included in several herbals as *Succus conii*, described as a narcotic, analgesic, anti-aphrodisiac, and anticancer agent, despite its poisonous nature. One time, It was listed in the British Pharmacopoeia and the British Pharmaceutical Codex as sedative and antispasmodic. Because of this property it was recommended as an antidote to strychnine (Grieve 1995–2004). Moreover, it was speculated that by modifying the structure to remove some of the toxic properties (teratogenic effects) (Lopez, Cid and Bianchini 1999, 841) it could be obtained for surgical purposes a substitute for curare (Binev 2013, 896-897).

Other pharmacological applications of some important plant-derived alkaloids are: cocaine (analgesic, narcotic, local anesthetic), hyoscyamine (anti-cholinergic), morphine (analgesic), hyoscine (sedative, anti-cholinergic) (Srivastava and Srivastava 2013, 216).

Herbal mixture – "Spice" - New Chemical Drugs and unidentified compounds

The use of legal, legal and natural alternatives has become a worrying global phenomenon in recent years. The fight against the illicit drugs traffic and consumption is a really complex social national and international preoccupation (Buzatu 2012, 20). The current concern for users, parents and professionals working with people using and abusing psychoactive substances is directly related to the identification of their effects and how they can have a negative impact on the body and the mind (Carey, Drug Education Officer Substance Misuse Service 2009, 3).

The ingredients in ethno-botanical products sold have three main sources: 1) psychoactive ethnobotanic herbs, 2) chemicals used primarily for research purposes, and 3) compounds that are gelling agents used to bind certain products.

Plants with psychoactive components used in the natural form are mainly with mild action but when certain alkaloids are extracted and used further result compounds comprised in the four classes of psychoactivity: hallucinogens, stimulants, anticoagulants and aphrodisiacs.

A psychoactive substance is a drug (any substance that changes the way the body and mind feels or believes), not controlled by legal acts and documents (Misuse of Drugs Act 1977-1984, Criminal Justice (Psychoactive Substances) Act 2010), which is legal to be detained and used. Most psychoactive substances are regulated by statutory acts that make their sale, supply and consumption unlawful. Many suppliers and manufacturers sell these products as "bath salts" or "incense", a way they can avoid the law. Psychoactive substances are sold with some ingredients that are not listed. The high level of danger of these substances is a cause for concern worldwide because they can cause serious effects on human health (Buzatu 2015, 5).

For example, "K2" and "Spice" are relatively new drug classes that have emerged recently (produced since 2004 by "street chemists") on the market as alternatives to marijuana. The Internet facilitates the purchase of these available drugs, which are sold in several stores under the guise of harmless products, such as herbal blends, incense or odorants (Buzatu 2015, 7, 17). Although the labels on the package indicate "forbidden", the number of poisoned people enrolled in emergency departments has increased dramatically (European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), 2009). In Europe, there has also been an increase in interest in Spice drug use (Jack 2009). Also in Europe in 2009, some countries (Austria, Germany, France, Luxembourg, Poland, Lithuania, Sweden, England and Estonia) introduced all products containing synthetic cannabinoid compounds under the Narcotics Law, so they were no longer accessible on-line or in specialized shops. The lack of validated and standardized testing procedures as well as the endless supply of such drugs are the main causes for researchers' difficulties in fully characterizing the clinical consequences associated with the use of Spice drugs. While precise chemical composition and toxicological analyzes of Spice drugs remain to be determined, there is evidence to identify several synthetic cannabinoids as main agents responsible for adverse physical and psychoactive effects (Seely, Lapoint, Moran and Fattore 2012, 234). Chromatography is a powerful instrumental technique in the analyses of selected NCE and represents the central axis of phytochemistry and the key to obtaining pure compounds for the purpose of elucidating the chemical structure, for

pharmacological testing, or for the development of compounds with therapeutic value (Marston 2007, 2785).

In 2009, Auwärter mentions that in order to demonstrate pharmacological activity and to obtain positive blood and urine samples, two of the authors have experimented the use of ethnobotanics. The two smoked a cigarette containing 0.3 g of "Spice diamond" and then took samples of blood and urine (Auwärter, Dresen, Weinmann, Muller, Putz and Ferreiros 2009, 1). Ten minutes after consumption, the first observable effects were eye reddening, pulse increase, xerostomia, and a mood and perception impairment. The effects were maintained for at least 6 hours with a slight attenuation. Some minor effects also occurred the following day. The observations were similar to those reported on blogs by other consumers and so the conclusion was one that aimed to confirm the presence of active pharmacological compounds. Following the application of herbicidal preparations such as Spice silver, Spice gold, Spice diamond, Smoke, Sence, Skunk and Yucatan Fire, qualitative drug analysis routines was done (GC-MS, LC-MS/MS).

These analyzes did not reveal the presence of any illegal drug or known pharmaceutical ingredient. Only α-, β- and γ-tocopherol as well as a series of phytosterols have been identified (Figure 2). Three abundant signals with unknown mass spectra were identified in GC-MS analysis instead. Two compounds had the same nominal molar mass (332 amu). The third compound was identified by a Frankfurt laboratory as JWH 018, a canabimimetic aminoalkyl indole with a 4-fold greater affinity for the CB1 receptor and a 10-fold greater affinity for the CB2 receptor relative to Δ9-tetrahydrocannabinol (THC). The cannabinoid CB1 receptor is mainly found in the central nervous system and CB2 in the peripheral system (Ameri 1999, 1). In the first two compounds, spectrophotometric investigations revealed phenolic chromophores. Other instrumental techniques have reached the molecular formula $C_{22}H_{36}O_2$. Compound 2 proved to be a homologue of a non-classical cannabinoid called CP 47,497 (fourth compound). Finally, it was shown that the first compound was in fact a trans-diastereomer of compound 2. Compound 4 in qualitative terms appeared to exhibit pharmacological characteristics similar to THC. In the preparations called Smoke, Skunk, besides JWH 018, high amounts of oleamide were identified. Ingestion of oleamides induces behavioral responses similar to cannabinoids (Leggett, Aspley, Beckett, D'Antona, Kendall and Kendall 2004, 260).

Compound 2 was also detected in blood samples (after consumption of Spice diamond). In these samples, 11-nor-9-carboxy- Δ 9-THC (the major oxidative metabolite of THC) was not shown.

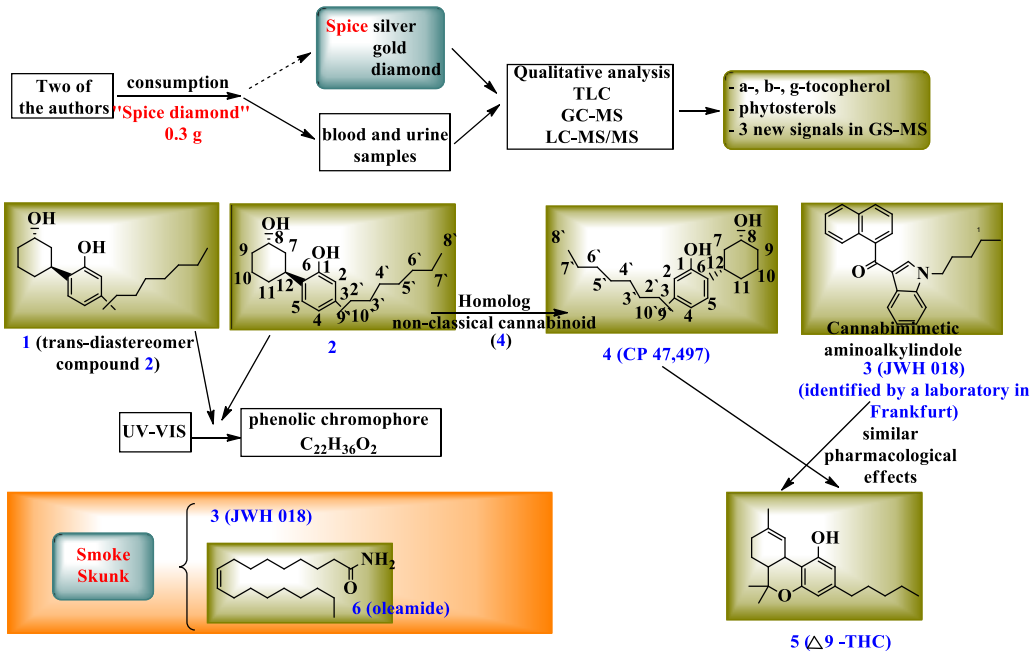


Figure 2. Experimental details to highlight some existing compounds in Herbal mixtures (Spice) (Mardare (Balusescu), Olariu and Arsene, 2017, 2016)

The final conclusion of the study by Auwärter, Dresen, Weinmann, Muller, Putz and Ferreiros 2009 is related to the fact that the chemical species identified with 2 and 3 represent strong agonist cannabinoid receptors that may be responsible for cannabis-like effects. There is currently no knowledge of the metabolite of these compounds. Some metabolites may be toxic and/or pharmacologically active. It is believed that there are hundreds of other compounds with cannabinoid receptor activity and therefore it is possible to assume that other substances will be on the market soon, substances that will cause great problems.

Conclusions

Plants have almost unlimited ability to synthesize secondary metabolites. Bioactive compounds in plants are means of potentiating drugs and can be so important for human health but can also cause toxic effects under certain conditions.

Essential plant compounds used as isolated compounds or as extracts, called the Novel Chemical Entities (NCE), offer unlimited opportunities and innovative leads for new drugs.

The use of legal drugs containing psychoactive substances (hallucinogens, stimulants, anticoagulants and aphrodisiacs) has been a worrying global phenomenon in recent years.

The recent emergence of relatively new drug classes "K2" and "Spice" as an alternative to marijuana on the market, and the continuous change in their composition is an important scientific burden for various laboratories.

It is absolutely necessary to identify the effects of drugs and how they can have a negative impact on the body and the mind.

There are lacking validated and standardized testing procedures to allow for the full characterization of drugs and the clinical consequences associated with drug use.

There are still inconsistencies in the legislative field and the incontestable evidence demonstrating the toxic nature of the abuse of these potential drugs.

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