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DETECTION OF ADULTERANTS IN HERBAL FORMULATIONS: A REVIEW

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ABSTRACT

The aim of this review is to determine the most commonly found adulterants in herbal formulations and effective methods developed for the detection and quantification of the adulterants. Weight loss formulations, dietary supplements, sexual performance enhancers, skin whitening agents and many more herbal medicines as stated used for therapeutic purposes are found contaminated with heavy metals, synthetic drugs, and radioactive substances, biological/microbiological and undeclared substances. More often, manufacturers resort to addition of adulterants to increase the efficacy of these medicines, however, most of these adulterants pose health risks to consumers. The techniques involved in the detection depends on the type of adulterants present in the formulation. Several methods based on chromatography, mass spectroscopy, spectroscopy, capillary electrophoresvis, and voltammetry have been reported over the years. Detection based on species specific variation has been a major breakthrough in the investigation of similar plant materials disguised as genuine plant products. This review highlights different techniques of detection of adulterant in herbal formulations.

Keywords: Herbal Formulations, Plant materials, Adulterants, Heavy metal, Detection, etc.

Introduction

The quality of the raw drug and formulations are compromised by the substitutions and adulteration of the raw herb. The adulterants vary in their origin and can include heavy metals, microbial / biological, radioactive substances, synthetic substances and undeclared substances [1,2,4]. Adulterants may also involve chemical ingredients and prescription drugs which pose a threat to health [16]. Weight loss formulations are adulterated with synthetic substances mainly sibutramine based on several studies [8,12,13,28] and sexual performance enhancers are often adulterated with sildenafil and similar compounds [10]. Instances of severe distress and even death have been reported which were caused by the intake of adulterated weight loss herbal formulations [11]. In such cases, the herbal medicines are often omitted from the list of evidence as they are considered 'natural' and remain undetected [14,15].

Chemical methods such as TLC, HPTLC MS, LC MS, GC FID, NMR, HPLC etc. and in combination are used for the authentication of botanical ingredients and commercial herbal products by various countries [40]. Chromatographic techniques coupled with

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mass spectroscopy have proven to be effective in the detection of synthetic and undeclared adulterants. Simultaneous toxicological analysis and DNA metabarcoding screened the presence of adulterants that can adversely affect the cardiovascular health of a person [33]. DNA based authentication of the commercial herbal product globally widespread revealed that in 27% of these products ingredients tested against labelled or claimed ingredients [41]. Microscopy is also considered as a rapid and cost effective method for the detection of adulterants based on morphological features with the raw samples [42].

Adulterants

Several herbal products are available in the market in the form of weight loss formulations, blood pressure lowering agents, lipid lowering agents, skin whitening agents and sexual performance enhancing agents [1,4]. These herbal formulations are often contaminated with undeclared synthetic drugs, steroids and toxic substances. Heavy metal such as Arsenic was detected in formulations used in cancer cure and mercury in skin whitening creams (1). Toxic natural substances, such as β Asarone, Xanthotoxin, Berberine, Benzyl cyanide and Digitalis glycosides which are carcinogenic and mutagenic were detected in herbal medicine[1]. Synthetic adulterants were added to improve the efficacy of herbal medicine. They belonged to different categories such as analgesics, antibiotics, antidiabetic and antiepileptic drugs, aphrodisiacs, hormones and anabolic drugs[2]. Bogusz et al.. detected benzodiazepines and tricyclic antidepressants, cyproheptadine in weight gain formulations and ibuprofen and dipyrone in rheumatism cure capsules[1]. A list of most common examples of adulterants used in the respective categories are provided in Table:1 (28, 34, 35, 36, 37).

Table: 1 Categories of Adulteration

Type of Adulterant	Adulterant
Heavy metal	Lead, Arsenic, Mercury, Cadmium, Aluminum, Nitrate and nitrite, Calcium, Copper, Iron, Potassium, Sodium
Microbial/ Biological	*Aspergillus flavus, Aspergillus funigatus Astralagussarcocolla, Coliform* and fecal coliform, *Bacillus cereus, Aeromonashydrophilia, Shigella spp., Enterobacter sp., Escherichia coli, Vibrio fluvialis.* Earthworm, Amoeba, Moulds, Protozoa, Yeasts, Nematode. Viruses. Insects.
Radioactive substances	Cs- 134, Cs - 137
Synthetic drugs	Tramadol, Caffeine, Fluoxentine, Rizatriptan, Venalafaxine, Methadone, Gibenclamide, Sibutraminehydrochloride, Sildenafil, Fenproporex
Undeclared chemical substances	Corticosteroids NSAIDS Benzodiazepines

Techniques and Analysis

In 2002, Bogusz et al.. examined 247 market samples of herbal formulations in Saudi Arabia, for presence of heavy metal using Inductively Coupled Plasma blended in herbal formulations [17]. Viana et al.. in 2013, described capillary zone electrophoresis with capacitive coupled contactless detection for the hypoglycemics and quantification of pharmaceutical adulterants present in herbal formulations used in

Mass Spectrometry (ICP MS), Gas chromatography Mass spectrometry (GC MS) for detection organic substances and microbiological examination for the detection biological contaminants[1]. In order to detect synthetic drugs such sildenafil, tadalafil, testosterone, glibenclamide and for monitoring multiple drugs within a short span of time, Bogusz et al.. in 2006, suggested the use of Liquid Chromatography Electron Spray Ionization (LC ESI MS)as an extension of GC MS for screening with a limit of detection 5pg to 1ng [2]. In 2009, Chen et al.. described Triple Quadrupole Ion Trap (QTRAP LC MS MS) on multiple reaction monitoring mode as a rapid, selective and sensitive screening method for synthetic adulterants such as glibenclamide, sibutramine hydrochloride, and sildenafil [4]. LC-MS/TOF (time of flight) was suggested as a reliable technique for the detection of steroids and anti-inflammatory drugs present in herbal formulations by Savaliya et al.. in 2009 [7]. Carvalho et al.. in 2010, reported the use of Adsorptive Cathodic Stripping Voltammetry (AdCSV) for the simultaneous detection of seven benzodiazepines and amfepramone in a single run based on chemical variations [3].

A comparative study was conducted on the performance of High Performance Liquid Chromatography (HPLC) and High Performance Thin Layer Chromatography (HPTLC) in detection of sibutramine by Ariburnu et al.. in 2012. Both the methods provided accurate and sensitive data, however, HPTLC exhibited higher sensitivity and shorter analysis time [8]. In 2012, Phattanawasin et al.. developed a method based on TLC image analysis and TLC densitometry for the quantification of sibutramine hydrochloride in slimming agents [12]. Synthetic substances such as Caffeine, Clenbuterol, Nicotinamide etc. were detected and quantified using UHPLC/DAD (Ultra High Performance Liquid Chromatography Diode Array Detection) by Rebiere et al.. in 2012 [13]. A new approach for detection of pharmacological adulterants was reported by Carvalho et al.. in 2012. In this approach, capillary electrophoresis with contactless detection for seven drugs including amfepramone, sibutramine, fenproporex, fluoxetine, paroxetine, sertraline [5]. In 2012, Balayssac et al.., used a combination of (Nuclear Magnetic Resonance) NMR, MS, IR and UV, in formulations treating erectile dysfunction and detected the presence of analogues of sildenafil with no therapeutic effect [10].

In 2013, Schaffer et al.. assessed the potential of 2D GC for detection of damiana, a 'herbal high' treatment of diabetes [19]. Near Infrared Spectroscopy with PLS and MLR mode proved to be a reliable method, by da Silva et al.. in 2013, for the qualitative and quantitative analysis of sibutramine in herbal medicines [21]. Biswas et al.. in 2013, described the use of PCR RFLP (Polymerase chain reaction Restriction Fragment Length Polymorphism) of ITS amplified regions for the discrimination of plants based on species specific variation [18].

Screening of 188 dietary products using LC MS/MS by Kim et al.. in 2014, revealed the presence of 29 weight loss compounds [20]. Detection of dexamethasone using reverse phase high performance liquid chromatography (RPHPLC) was described by Sairam et al.. in 2014 [22]. Lv et al.. in 2015 developed an approach in which Thin-Layer Chromatography (TLC) followed by Surface-Enhanced Raman Scattering (SERS), used for the detection of ephedrine analogues and the results were confirmed by UPLC QTOF MS [23]. In 2016, Russo et al.., described LC/UV method for the simple and cost effective method for simultaneous screening and quantification of Fluoxetine, Tiratricol, Benfluorex and Pseudoephedrine [24]. Urumarudappa et al.. in 2016 described DNA barcoding validated by NMR spectroscopic techniques for the detection of presence of seven different families [38].

In 2017, Cebi et al.. described ATR FTIR (Fourier Transform Infrared Spectroscopy) combined with chemometrics for the detection of sibutramine in tea and coffee [25]. A study conducted by Maasecsu et al.. in 2017 using Raman and FTIR spectroscopy revealed that 22% of herbal medicines in the Romanian market claiming sexual enhancement were adulterated with sildenafil, tadalafil and phenolphthalein [26]. Shekari et al.. in 2018, described the potential of GC MS fingerprinting in detection of a variety of adulterants including synthetic substances and carcinogenic components present in slimming pills [27]. Examinationof 61weight loss drugs marketed in Iran by GC MS, revealed 72 % adulteration with synthetic drugs and was reported by Dastjerdi et al.. in 2018 [28]. A similar study conducted by Fakhri et al.. in 2018 on 15 slimming products with dual approach of LC UV (ultraviolet)and LC MS detected Phenolphthalein to be the most abundant adulterant, followed by it, caffeine, phendimetrazine, protriptyline[29]. DNA barcode signatures of nr-*ITS and psbA-trn*H were utilized for the identification of plant species by Kumar et al.. in 2018p39]. Other regions such as rbcL and Matk are also utilized for similar studies. In 2019, Khare et al.. established

the effectiveness of FTIR analysis in the detection of substances such as modafinil, salbutamol, phenolphthalein and caffeine [30]. In 2019, Freitas et al.. developed a portable method for the detection of presence or absence of sibutramine in food supplements. This method involves a simple sample preparation step, followed by which the sample undergoes a scan to provide the reliable results. The method is called Square Wave Voltammetric method [31].Salashour et al.. in 2020 described the potential of High-Performance Liquid Chromatography with Diode Array Detector (HPLC-DAD) and Gas Chromatography-Mass Spectrometry (GC-MS) techniques for the detection of undeclared adulterants including Caffeine, trimethoxyamphetamine, and vitamin E [32]. Techniques used in the detection of adulteration shows in the Table:2

Table 2: Techniques used in the detection of adulteration

Technique	Adulterants	References
ICP MS GC MS	Heavy metals: Arsenic, Mercury, Lead, Toxic substances: Benzodiazepines Organic substances	[1]
LC ESI MS-MS	Sildenafil, Tadalafil, Testosterone, Glibenclamide	[2]
QTRAP LC MS-MS	Glibenclamide, Sibutramine hydrochloride, Sildenafil	[4]
LC-MS/TOF	Dexamethasone diclofenac, piroxicam	[7]
Voltammetry	Benzodiazepines, Amfepramone	[3][6]
HPTLC	Sibutramine	[8]
NMR MS IR UV	Sildenafil analogue	[10]
TLC	Sildenafil hydrochloride	[12]
CE with contactless detection	Amfepramone, Sibutramine, Fenproporex, Fluoxetine, Paroxetine, Sertraline	[5]
UHPLC -DAD	Caffeine, Clenbuterol, Nicotinamide, Phenolphthalein, Rimonabant,Sibutamine, Didesmethylsibutramine, Synephrine, Yohimbine	[13]
Two-Dimensional Gas Chromatography	Damiana	[17]
PCR RFLP	Species specific variation	[18]
Capillary Zone Electrophoresis (CZE) With Capacitively Coupled Contactless Conductivity Detection	Metformin, Chlorpropamide, Glibenclamide , Gliclazide	[19]

LC MS-MS	Bisacodyl, Sibutramine & its derivatives, Ephedrine, Sennoside A & B	[20]
Near infrared spectroscopy	Sibutramine	[21]
RP HPLC	Dexamethasone	[22]
TLC-SERS	Ephedrine Analogues	[23]
LC-UV	Fluoxetine, Tiratricol, Benfluorex and Pseudoephedrine	[24]
DNA barcoding NMR	Differentiation between plants belonging to different families	[38]
FTIR-ATR	Sibutramine	[25]
Raman and FTIR spectroscopy	Sildenafil, Tadalafil Phenolphthalein	[26]
GC MS Fingerprinting	Phenolphthalein, amfepramone, caffeine and sibutraminepyrogallol, furfural	[27]
GC MS	Tramadol, Caffeine, Fluoxetine, Rizatriptan, Venlafaxine And Methadone.	[28]
LC-UV and LC-MS	Phenolphthalein, caffeine, phendimetrazine, protriptyline	[29]
FTIR	Modafinil, Salbutamol, Phenolphthalein Caffeine	[30]
Square-Wave Voltammetric Method	Sibutramine	[31]
HPLC-DAD and GC MS	Caffeine, trimethoxyamphetamine, vitamin E	[32]

Conclusion

The study has reviewed different detection methods employed by various research groups for the qualitative and quantitative analysis of different types of adulterants in herbal formulations. Detection has revealed the possibility of ingestion of toxic components by individuals unintentionally. Long term exposure to such drugs can lead to the development of irreversible medical conditions. Instances of death due to the consumption of adulterated formulations though rarely reported, occur and often are undetected due to the belief of being natural therefore assumed safe to consume. Moreover, the quantity of adulterants sometimes being negligible as compared to the active ingredient of formulation, conventional detection methods do not detect the adulteration or the lethal component in it.

Several synthetic substances, steroids and heavy metals can be detected with an integrated approach of chromatography and mass spectroscopy. Voltammetry based on site detection method which

is portable in nature can be used for detection of sibutramine has been reported. However, the method is time consuming and tedious and less reliable. More sensitive, cost effective, rapid and easy methods can be developed for the detection of other adulterants. DNA based techniques PCR, RFLP, Plant Barcoding and Meta genomics can be aided to differentiate between botanical adulterants and the genuine herbal constituents. The intensive research works conducted over the years indicate the urgent need for the development of a DNA based global authentication system for herbal constituents and formulations.

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