Current Clinical and Medical Education

Received 19 Mar 2024 | Revised 20 Mar 2024 | Accepted 28 Apr 2024 | Published Online 02 May 2024

vision publisher Medical Journals

Published By: Vision Publisher

ISSN 2942-0792

CCME 02 (5), 65-78

Metabolomics by Gas Chromatography-Mass Spectrometry (GCMS): Genotypes, Phenotypes and Secondary Metabolites

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Abstract : Metabolomics utilizing gas chromatography-mass spectrometry (GC-MS) is well-suited for the detection and quantification of small molecular metabolites (<650 daltons), such as small acids, alcohols, hydroxyl acids, amino acids, sugars, fatty acids, sterols, catecholamines, drugs, and toxins. Chemical derivatization is frequently employed to render these compounds gas chromatographically volatile. This section demonstrates how GC-MS metabolomics makes it simple to combine untargeted metabolomics for the discovery of new substances with targeted experiments for the absolute quantification of particular metabolites. In human body fluids (e.g., plasma, urine, or stool) samples, GC-MS may detect and semi-quantify over 200 substances each study. This is made possible by database annotations employing huge spectral libraries and established, standardized standard operating procedures. Like liquid chromatography-MS untargeted profiling (LC-MS), deconvolution software allows for the detection of over 300 more unknown signals that can be annotated using precise mass instruments and suitable data processing methods. As a result, gas chromatography–mass spectrometry (GC–MS) is an established technique that employs a variety of mass spectrometers, including traditional quadrupole detectors, target mass spectrometers, and precise mass instruments. Metabolomics using gas chromatography-mass spectrometry is covered in this unit. (i) Collecting data, (ii) checking for quality, and (iv) processing the data obtained from samples of mammals. The creation of secondary metabolites has sparked a lot of attention in the past few years, as has the prospect of improving that production through the use of tissue culture technologies. So far, there has been a good amount of published research and abstracts assessing the antioxidant, antimicrobial, and anti-diabetic properties of numerous plant-derived secondary metabolites. We looked at the bioactivity of eighteen different plant compounds, including flavonoids, alkaloids, and phytosterols. It was discovered that a total of eleven plants exhibited antioxidant, antimicrobial, and anti-diabetic secondary metabolite potential. Many plant parts, including roots, stems, leaves, fruits, and flowers, contain secondary metabolites that demonstrate bioactivity.

Keywords: GC-MS, Compound, Pathway Mapping, Multivariate.

Supplementary information The online version of this article (https://doi.org/xx.xxx/xxx.xx) contains supplementary material,which is available to autho-rized users.

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Introduction

There have been established protocols for the analysis of metabolites (such as sugars, amino acids, sterols, hormones [1], catecholamines, hydroxyl acids, fatty acids, aromatics, and many other primary metabolism intermediates) using gas chromatography-mass spectrometry (GC-MS), making it the most standardized method in metabolomics. It was in the 1970s when GC-MS initially achieved the exciting prospect of combining targeted analysis of certain compound classes into profiling ssays for vast swaths of metabolism. Since then, this concept has been used to improve the diagnosis of human disorders, with 140 patients or more involved. To highlight the requirement of identifying and quantifying all small molecules present in a particular biological setting, we now call such profiling "metabolomics" [2-4]. Cellular machinery can use such profiles as output in response to genetic or environmental perturbations.

Metabolomics: the bridge between genes and their manifestations in the body

The levels of metabolites, which are byproducts of cellular regulatory mechanisms, can be seen as the last reaction of biological systems to changes in genetics or the environment. Similar to the words "transcriptome" and "proteome," the "metabolome" refers to the collection of metabolites that an organism produces. The impartial simultaneous detection and quantification of plant metabolomes, however, has received little attention, in contrast to other functional genomics methods [5]. Not long ago, there wasn't enough analytical granularity to identify metabolite concentrations and identities on an individual basis; instead, most studies could only profile certain types of substances or fingerprint metabolic alterations. Before doing metabolomic analysis, it is necessary to thoroughly evaluate the procedures used for obtaining tissue samples, preparing samples for data gathering, and mining the results. This paper aims to clarify the differences between metabolic fingerprinting, metabolite profiling, and metabolite target analysis while also providing definitions for key words. For more than four decades, mass spectra and chromatographic retention times have been collected and made publicly available in libraries—most notably in the NIST 14 Mass Spectral Library collection of the U.S. National Institute of Standards and Technology (NIST)—under standardized conditions of 70 eV electron ionization energy. Other larger, less curated versions of this data can be found in places like the Golm repository, the open-access MassBank database, and the Wiley registry. Through the use of several literature sources, a database including the gas chromatographic retention properties of chemical compounds has been established. There are presently 42,888 compounds with 292,924 records of retention data in the NIST database for both polar and non-polar stationary phases. Retention volumes, retention times, linear indices, Kováts indices, and Lee indices are all part of the database. The initial version of this database, which covers non-polar stationary phases [7, 8], was made available in June 2005 through the NIST/US EPA/NIH Mass Spectral Database and is also accessible online through the NIST Chemistry WebBook.

MassBank was the initial online database to store mass spectra of tiny chemical compounds (<3000 Da) for use in the biological sciences. In January 2010, 16 research groups contributed 605 EI-MS, 137 fast atom bombardment MS, and 9276 electrospray ionization (ESI)-MS(n) data points from 2337 authentic metabolite compounds, 10,286 volatile natural and synthetic compounds from 11,545 EI-MS and 834 other-MS, and 30,45 ESI-MS(2) data points from 679 synthetic drugs. Nonstandardized, separate experimental conditions were used to examine the ESI-MS(2) results. The MassBank database is decentralized. Data is provided by each study group using their respective Internet-based MassBank data servers. By adjusting a few experimental parameters, users of MassBank can gain access to a subset of the data or all of the data. After optimizing the weighting exponents on peak intensity and the mass-to-charge ratio to the ESI-MS(2) data, the similarity score is computed using a weighted cosine correlation in a spectral search to return mass spectra that are comparable to a query mass spectrum [9, 10]. MassBank additionally offers a combined spectrum for every chemical, which is made by combining the evaluated ESI-MS(2) data on a single chemical subjected to varying collision-induced dissociation states. The accuracy of chemical compound identification has been enhanced by 21-23% at a similarity score of 0.6 by the use of data merging. As a result, chemical compound identification and experimental data release are two areas where MassBank shines. Beyond these standardized libraries, GC-MS possesses unique benefits that have earned it the title of "gold standard" in metabolomics [11]. This means that newer methods should be evaluated in relation to its ability to detect

metabolites with breadth, sensitivity, and specificity. Specifically, mass spectra recorded in big user libraries with standardized protocols for data acquisition, like the BinBase databases and the Fiehnlib libraries, can be used to improve the specificity of mass spectral matching, because electron ionization causes complex and rich fragmentation patterns [12].

When it comes to metabolomics, high-throughput, quick analysis is more important than using traditional, semi-automated methods for deconvolution of data from gas chromatography-mass spectrometry (GC-MS) investigations. Present research used three distinct deconvolution software packages: LECO ChromaTOF, AMDIS, and SpectralWorks AnalyzerPro, to handle data sets obtained utilizing GC with time-of-flight MS (GC-TOF-MS). We were interested in the adaptability and efficiency of these programs, as well as the level of detection, identification, and agreement of qualitative outcomes. Data from the examination of a test-mixture solution containing 36 endogenous metabolites with a wide range of relative concentration ratios were used for comparisons [13]. Variations in component identification rate and deconvolution accuracy were noted. After deconvolution, the mass spectra were run via the AMDIS Search tool. They were compared to libraries that the author had built themselves, which contained both the mass spectra and the retention indices of derivatives of a set of metabolites. Analyte identifications were based on spectrum similarities and retention indices. Both the quantity of components discovered and the quality of the results varied significantly across the three applications. While AnalyzerPro did not generate many false negatives, AMDIS and ChromaTOF did. Component width stands out as the key parameter in these three software packages when it comes to forecasting the deconvoluted result's correctness.

As a result, peak selecting is done in tandem with real mass spectral deconvolution, which, in contrast to LC-MS methods, uses MS and data dependent MS/MS fragmentations together to produce purified mass spectra, rather than separately. Metabolomics have made good use of the freely accessible automated mass spectrum deconvolution software (AMDIS) for GC-MS since its release in 1998. Efforts to untargetedly couple ionization and fragmentation in LC-MS have recently begun, for instance using SWATH approaches. However, as of right now [14], neither commercial nor open-source LC-MS software can purify the mass spectra of co-eluting mass spectra in GC-MS (e.g., AMDIS nor ChromaTOF).

People have always looked to the natural world to provide for their most fundamental need, including food, housing, clothes, transportation, fertilizers, flavors, scents, and medical care. Both traditional and modern medical practices rely heavily on plant-based remedies. There are a number of factors that contribute to the significance of medicinal plants, such as the low cost and limited accessibility of Western medicine and the widespread belief in herbal therapy as a result of its efficacy.

As a whole, people are starting to "return to nature" by using herbal remedies instead of synthetic ones. Medications originating from plants have played an important role in human healthcare for a very long time. Medicinal plants are defined as plants that contain chemicals that have therapeutic potential or that are building blocks of novel semi-synthetic chemo drugs by the World Health Organization. For many generations, people have relied on natural remedies. Herbal remedies are abundant in the folk medicine practices of nearly every culture on Earth. Traditional medicinal practices rely heavily on plant-based remedies. The synthetic chemistry of pharmaceuticals and antibiotics have come a long way, but plants are still a key source for many of the drugs used to treat human diseases. Research in the fields of medicine and pharmacology has brought medicinal plants into the spotlight by illuminating the functions of their active ingredients and providing more details on how they affect both humans and animals. Any plant that contains chemicals with therapeutic potential or that has precursors for the chemopharmaceutical synthesis is considered a medicinal plant. Plants have long been used for a variety of medicinal purposes, including the treatment of infectious disorders (e.g., diarrhea, fever, cold), the prevention of pregnancy, and the maintenance of good oral hygiene. Traditional medicine also makes use of a number of psychotropic compounds derived from plants. There is a wide range of recognized therapeutic qualities produced by traditionally used medicinal herbs.

In many third world nations, traditional herbal medicine is the backbone of healthcare. Natural goods with therapeutic characteristics have been traced back to the extensive usage of herbal remedies and health care

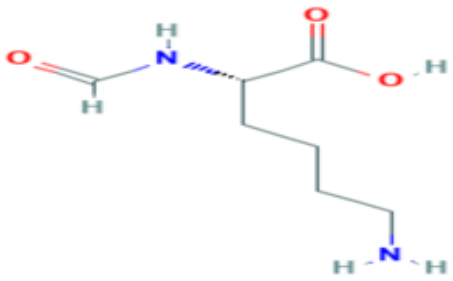
preparations, which are derived from commonly used traditional herbs and medicinal plants. These remedies and preparations have ancient descriptions in writings like the Vedas. Using a combination of natural items to treat illnesses has shown some intriguing results, such as the poly-pharmacological application of plant extracts and synergistic effects. Thousands of plant species in India have been traditionally used for medical purposes, and the practice of combining various plant parts to treat individual diseases dates back to ancient times. An estimated 70,000 plant species have a history of medicinal usage, according to Purohit and Vyas. If you want to live a long, healthy life, Ayurveda is the way to go. Not only is it the most holistic medical system now available, but it is also the oldest (almost 5,000 years). This approach has its roots in the Vedas, specifically the Atharvaveda [17]. Traditional Ayurvedic medicine, sometimes known as Ayurveda, is an alternative medical system with its roots in India. In India, the Vedic period saw the publication of the first medical texts. Ayurveda records the medicinal properties of several native plants. More than 700 plants are mentioned in the Susruta Samhita and the Charaka Samhita, two important texts on traditional medicine from this period. In the decades that followed, Ayurvedic doctors perfected a number of surgical techniques and pharmacological concoctions for the treatment of a wide range of diseases. Herbal remedies are gaining popularity as a modern medicine option due to its efficacy in treating a wide range of medical conditions. In addition to being easily accessible and inexpensive, they are also not known to have any significant negative side effects. Synthetic pharmaceuticals are prohibitively expensive, insufficient, and rife with adverse effects and adulterations in underdeveloped nations. This led to the development of regionally specific therapies [18]. According to the World Health Organization, herbal medicine is the mainstay of healthcare for over 80% of the global population in underdeveloped nations. Therefore, it is necessary to seek out plants that possess medicinal properties. A lot of people throughout the world have started talking about traditional medicine recently. Scientific investigations into the potential therapeutic uses of many plant species now accepted as medicinal herbs are numerous. Acquiring chemicals derived from plants is becoming more challenging due to factors such as the rapid disappearance of natural habitats for medicinal plants and environmental and geopolitical instability [19]. As a result, businesses and researchers are starting to wonder if cell cultures could be a viable alternative to traditional methods of extracting plant medicines. It is well-known that phyto-compounds are essential for plant adaptation to their environments, but they are also a promising new class of medicines [20]. The empirical medical system owes its origins to the utilization of plant and extract-based medicinal products, which laid the groundwork for contemporary therapeutic sciences. As a result, these medicinal chemicals are being extracted from plants and tested on a number of different plant species (Table 1).

Table 1. GCMS analysis of ethanolic leaves extract of *Cordia myxa*

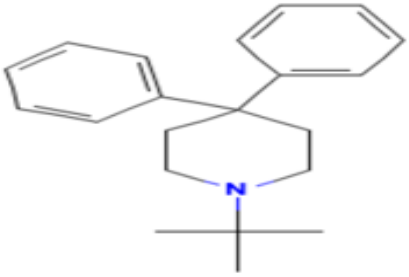
Compound	Molecular Formula	Molecular Weight
Diethyl cyclohexylphosphoramidate	$C_{10}H_{22}NO_3P$	235.26 g/mol
Formyl-L-lysine	$C_7H_{14}N_2O_3$	174.20 g/mol
1-tert-Butyl-4,4-diphenylpiperidine	$C_{21}H_{27}N$	293.4 g/mol
D-Glucose, 4-O-beta-D-galactopyranosyl	$C_{12}H_{22}O_{11}$	342.30 g/mol
Luteolin 6,8-di-C-glucoside	$C_{27}H_{30}O_{16}$	610.5 g/mol
2,5-Dimethyl-4-hydroxy-3(2H)- furanone	$C_6H_8O_3$	128.13 g/mol
phenylcyclopropyl)methanamine	$C_{10}H_{13}N$	147.22 g/mol
Dodecanoic acid, 3-hydroxypropyl ester	$C_{15}H_{30}O_3$	258.40 g/mol
6,9,12,15,18,21-Tetracosahexaenoic acid,	$C_{24}H_{36}O_2$	356.5 g/mol
12-Methyltridecanal	$C_{14}H_{28}O$	212.37 g/mol
Octanal, 7-methoxy-3,7-dimethyl	$C_{11}H_{22}O_2$	186.29 g/mol
Methyl 4,8,12-trimethyltridecanoate	$C_{17}H_{34}O_2$	270.5 g/mol
Ethyl 2-acetyldecanoate	$C_{14}H26O3$	242.35 g/mol

5-Hydroxymethylfurfural	**$C_6H_6O_3$**	**126.11 g/mol**
3-Methoxy-1-hydroxymethyl-adamantane	**$C_{12}H_{20}O_2$**	**196.29 g/mol**
4-O-alpha-D-Glucopyranosyl-moranoline	**$C_{12}H_{23}NO_9$**	**325.31 g/mol**
2-Cyclohexylpiperidine	**$C_{11}H_{21}N$**	**167.29 g/mol**
3-O-Methyl-d-glucose	**$C_7H_{14}O_6$**	**194.18 g/mol**

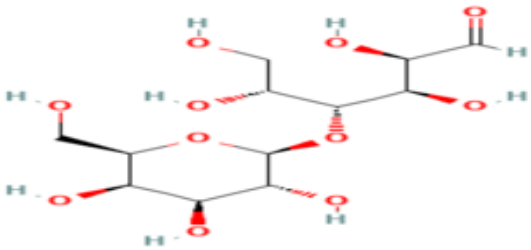
Diethyl cyclohexylphosphoramidate



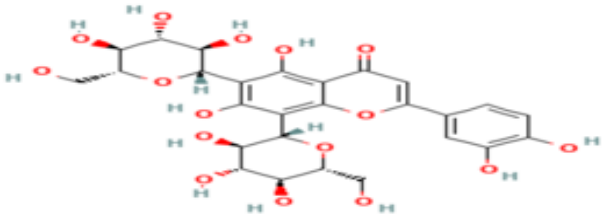
Formyl-tert-Butyl-4,4-



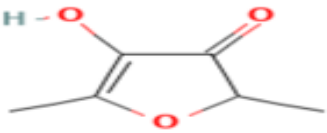
diphenylpiperidine l-L-lysine



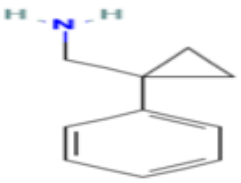
D-Glucose, 4-O-beta-D-galactopyranosyl



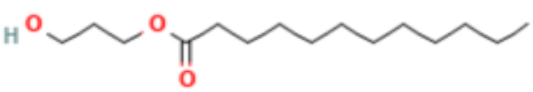
Luteolin 6,8-di-C-glucoside



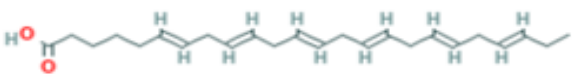
2,5-Dimethyl-4-hydroxy-3(2H)- furanone



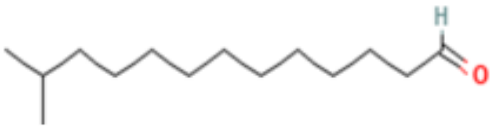
phenylcyclopropyl)methanamine



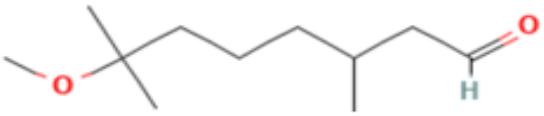
Dodecanoic acid, 3-hydroxypropyl ester



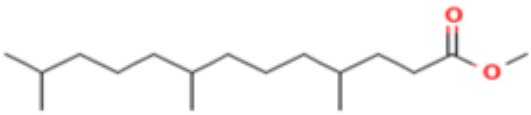
6,9,12,15,18,21-Tetracosahexaenoic acid



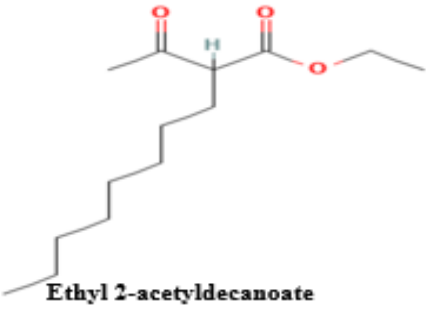
12-Methyltridecanal



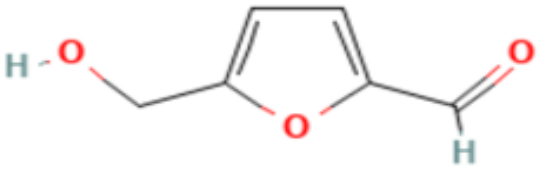
Octanal, 7-methoxy-3,7-dimethyl



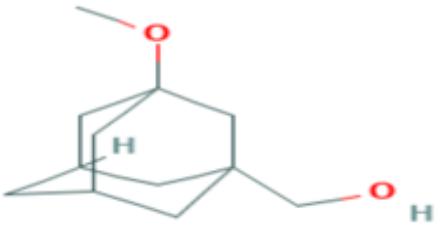
Methyl 4,8,12-trimethyltridecanoate



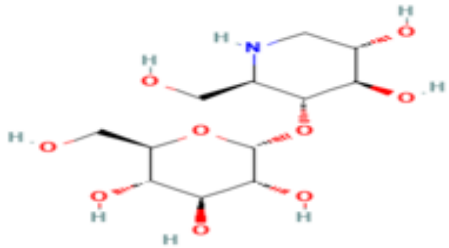
Ethyl 2-acetyldecanoate



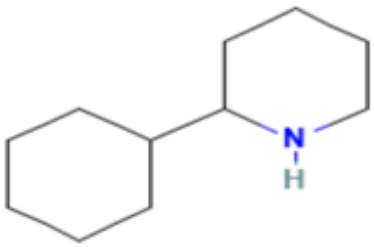
5-Hydroxymethylfurfural



3-Methoxy-1-hydroxymethyl-adamantane



4-O-alpha-D-Glucopyranosyl-moranoline



2-Cyclohexylpiperidine

Extracellular Molecules:

The metabolic process yields metabolites as its byproducts and intermediates. Small molecules are typically the only ones referred to as metabolites. Among metabolites' many roles are those of fuel, structure, signaling, enzyme stimulation and inhibition, catalytic activity (often in the role of a cofactor), defense, and interaction with other microbes. There is a wide variety of organic compounds produced by plants, and most of them don't seem to have any role in the formation or growth of the plant itself. The distribution of these chemicals, which are often known as secondary metabolites, might vary within certain taxonomic groups in the plant kingdom [21]. Recent years have seen a surge in interest in secondary metabolites due to their growing economic significance, with a focus on the potential to modify the synthesis of bioactive plant metabolites through the use of tissue culture technologies. For both the methods of multiplication and the extraction of secondary metabolites, sterile conditions can be routinely used to establish plant cell and tissue culture technology from explants, such as stems, roots, leaves, and meristems.

There have been reports of commercial and medicinal plants being able to produce secondary metabolites in vitro through the use of plant cell suspension cultures. Secondary metabolites are not immediately lethal, but their lack can harm an organism's fertility, beauty, or survivability in the long run, or cause no change at all. This is in contrast to primary metabolites, which cause death right away. Typically, these are only applicable to a small subset of taxa within a given phylogeny [22]. Among their many functions, they are crucial in herbivory defense and other forms of interspecies protection. Biosynthetic pathways lead to the formation of secondary metabolites, which include a diverse array of active chemicals. Among plants, they are rarer and less common. For the same plant species, their number and quality can change depending on where they're grown. Specialized cell types at different phases of development often produce them, therefore they accumulate in lower amounts.

The medicinal plants include a wealth of secondary metabolites, a diverse set of chemicals that have found widespread usage in the drug and pharmaceutical industries. These metabolites include alkaloids, glycosides, amines, insecticides, steroids, flavonoids, and related compounds. Some secondary metabolites in plants exist in inactive precursor forms and are activated in reaction to tissue injury or pathogen attack; others, known as constitutive metabolites, are present in healthy plants in their physiologically active forms. Alkaloids, steroids, tannins, flavonoids, resins, fatty acids, etc. are only a few examples of the secondary compounds found in plants that might have favorable medical effects when combined. Terpenoids make up 33,000 of the total secondary metabolites listed in the natural products dictionary, whereas alkaloids account for 16,000 and flavonoids for 8,182. Not only are they engaged in fundamental metabolism, but they also play an ecological function by helping plants withstand biotic and abiotic stressors. number 8. Cell coloration in flowers and seeds, which attract pollinators [23], seed dispersers, and is involved in plant reproduction are all processes that involve certain secondary metabolites, like flavonoids. Secondary metabolites found in plants also have medicinal qualities that can benefit people's health [24]. A common technique to categorize secondary metabolites in plants is by the metabolic routes they follow during biosynthesis. Flavonoids, steroids, alkaloids, and terpenoids are the four main groups into which these big molecules fall.

Alkaloids:

Heterocyclic nitrogen molecules are known as alkaloids. They originate from amino acids like tryptophan, tyrosine, and lysine, which are examples of primary metabolites. As a result of their complicated chemical structures, alkaloids can have lengthy production routes. For almost three thousand years, people have turned to alkaloids for relief from a variety of symptoms, including fever, insanity, snakebite, and a sore throat. There is a class of chemical substances in nature called alkaloids [25]. Alkaloids are the most abundant group of secondary plant metabolites; there are an estimated 5,500 species in this group. Their pharmacological effects have led to their usage in medicine, recreational drug use, and entheogenic ceremonies.

Several viruses have been found to be inhibited by papaverine, a benzylisoquinoline alkaloid; indoquinoline alkaloids from Cryptolepsis sanguinolenta have demonstrated efficacy against certain gram-negative bacteria and yeast. The alkaloid quinine is well-known

for its ability to inhibit the parasite's ability to cause malaria. There is a lot of evidence in the literature that plant alkaloids are biologically active. Additionally, many alkaloids have a harmful effect on other creatures. Some believe that alkaloids, as secondary metabolites, help plants ward against herbivores and diseases [26]. About 12,000 alkaloids have been used as medicines, stimulants, drugs, and poisons because of their strong biological activity. Almost from the dawn of civilization, plants that contain alkaloids have been used as dyes, species, medications, or poisons.

Flavonoids:

A class of chemicals known as flavonoids is a class of polyphenols. Everything from fruits and vegetables to nuts and seeds to stems and flowers as well as tea, wine, propolis, honey, and photosynthesizing cells contain them. They have a long history of use due to their reputation for medicinal characteristics and their pivotal part in effective ancient medical therapies. They have powerful anti-cancer properties and are free radical scavengers and powerful antioxidants that are water-soluble. They have antibacterial, anti-inflammatory, antispasmodic, and anti-allergic properties, and they enhance aquaresis. There was some evidence that flavonoids could reduce blood pressure and increase blood circulation. The biological action of flavonoids includes the inhibition of several enzymes, including phosphodiesterase, ATPase, aldose reductase, xanthine oxidase [27], cyclooxygenase, lipoxygenase, and others. Various hormones, including thyroid hormone, estrogen, and androgens, are regulated by them as well. Their anti-inflammatory effects have been shown in both the proliferative and exudative stages of inflammation. Plants generate flavonoids at specific locations; these compounds are responsible for many plant functions, including color, fragrance, fruit (which attracts pollinators and helps spread the fruit), seed and spore germination, and seedling growth and development. The unique UV-filtering properties, signal molecules, allelopathic substances, phytoalexins, detoxifying agents, and antimicrobial defense compounds of flavonoids help plants withstand a variety of biotic and abiotic stresses.

Phytosterols:

A hydroxyl group at carbon-3, typically in a beta configuration, and branching side chains from eight to ten or more carbon atoms at carbon-17 are characteristics of sterols, one of several main classes of steroids. Their distribution is extensive across the animal and, in especially, plant worlds. They are essential components of membranes and play a pivotal part in the biosynthetic pathways that produce steroidal species, in addition to their structural responsibilities. The process of biosynthesis in plants begins with the sterols. The manufacture of plant steroids begins with the phytosterols, which are known to be present in all higher plants. Because humans are unable to produce them, they must get them through food sources [28]. Several investigations have shown that their serum cholesterol level has dropped. Industrially significant steroid chemicals, pesticides, antioxidants, and anticancer medications are derived from plant sterols. Some of the sterols that have been documented are those that have been extracted from different plants. Aside from β-sitosterol, β-stigmasterol, lanosterol, and campesterol are among the most frequently isolated sterols from higher plants. Other researchers also found β-sitosterol, campesterol, and stigmasterol in higher plants, although they separated these chemicals less frequently. There is mounting evidence that phytosterols have multiple beneficial effects, including reducing inflammation and cancer. Specifically, phytosterols have been found to be effective against ovarian, lung, stomach, and estrogen-dependent breast cancer in humans.

Manufacturing Secondary Metabolites and New Process Improvements:

Pharmacists, food scientists, and chemical engineers have a one-of-a-kind resource in plant secondary metabolites. Additionally, they supply unique resources that are utilized in several fields. A potential alternative to chemical synthesis or plant extraction for creating metabolites that are difficult to obtain has been established through plant cell culture. This offers an option to directly extracting chemicals or derivatives from plants, as well as to chemical synthesis for comparable purposes. Plant cell culture technology has come a long way in decades, but there are still numerous biological and biotechnological obstacles to overcome before it can fully produce plant secondary metabolites. Plant cell cultures have a poor output of secondary plant metabolites, which is a big problem. Some strategies for culture production of metabolites have been developed to increase the yield of plant secondary metabolites. These strategies include treating plants with different elicitors, signal compounds, and biotic stresses. Plant

secondary metabolites primarily serve to protect plants from insect attacks, herbivores, and pathogens, as well as to survive other biotic and abiotic stresses. In fact, many of these treatments do a good job of encouraging the in-vivo and invitro development of many different secondary plant metabolites. Nevertheless, productivity is still not up to par when it comes to industrial use.

The Bioactivity of Secondary Plant Metabolites:

Function as an Antioxidant:

It has been found that antioxidants can protect against free radical oxidative damage. Defense systems in all living things scavenge and stabilize free radicals, but when the rate of free radical synthesis surpasses what is considered a healthy level, oxidative stress is triggered, which can damage cellular components such as DNA, lipids, and proteins. Many medication formulations based on antioxidants are utilized for the prevention and treatment of diseases whose processes entail oxidative stress. Finding naturally occurring antioxidants to utilize in food, cosmetics, or medicinal materials has become much more popular as a result of the ban on manufactured antioxidants owing to their carcinogenicity. Natural antioxidants found in a variety of medicinal plants may be able to neutralize free radicals in the body, according to certain theories. Natural foods' curative properties in neutralizing free radicals have, thus, garnered a lot of attention as of late. For this reason, there has been a lot of buzz about using biological systems to scavenge free radicals and adding antioxidants derived from natural sources into food [31]. As an alternative to synthetic antioxidants, which can be hazardous and poisonous, they may give a safe alternative. Research has shown that certain plant secondary metabolites can act as antioxidants.

Limonia acidissima:

Limonia has flavonoids, according to preliminary research. Three significant compounds, namely kaempferol, quercetin, and lutein, are detected by thin layer chromatography. This GC-MS analysis uncovered a chemical that is quite close to the flavonoids 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one. When compared to other plant parts, the leaf of Limonia acidissima demonstrates the highest antioxidant potential. By using the DPPH test, the IC50 value was found to be 50.03µg /ml. The activity of lipid peroxidation (LPO) was found to be 6.045 ± 0.55 µmols MDA g-1DW13 in the leaves of L. acidisssima.

Murraya koenigii:

Kochia murrayi Three compounds—Kaempferol, quercitin, and luteolin—were identified by TLC analysis. Its greatest antioxidant capacity was demonstrated by the DPPH and lipid peroxidation assays on its leaf. By DPPH assay, the IC50 value was found to be 49.86 µg/ml. The plant leaf had an activity level of 23.715µmols MDA g-1DW, which is indicative of lipid peroxidation.

Duranta erecta:

Benzene 1,4-diol, Trans-cinnamic acid, and thirteen other components were identified in the GC MS analysis of Duranta erecta. dicarboxylic acid 1,2-benzene 2H-1-Benzopytran-2-one, 6,7-dimethoxy-, 2(4H)-Benzofuranone, etc. are among the several chemicals that are comparable to flavonoids. D. erecta root extract has substantially greater DPPH and peroxidize antioxidant activity than D. erecta leaf and stem extracts. In the lipid peroxidize method, the leaf exhibited a stronger antioxidant potential with a value of 38 µmols MDA g-1FW, and the IC 50 value was found to be 40.97µg/ml.

Petrea volubilis L.:

The DPPH assay found an IC50 of 36.85 µg/ml in the root of P. volubilis, indicating its best antioxidant capacity, while the leaf demonstrates the highest lipid peroxidation assay value of 24.955 µmols MDA g -1FW. Research shows that of all the plants tested, the leaf of P. volubilis had the highest level of antioxidant activity.

Rumex vesicarius:

Antioxidant activity is enhanced by R. vesicarius methanolic extracts. The leaf displayed superior antioxidant activity as measured by the DPPPH test. Various other tests, including the peroxidase assay, the ferric reducing ability of plasma (FRAP) assay, and the lipid peroxidation assay, all indicated that the leaf has the highest antioxidant capability of the entire plant. The IC50 value for the DPPH activity is 174.91 ± 17.96 µg/ml. The leaf's methanolic extract reduced the MDA level to 6.48 ± 2.0 µM MDA-1DW, which was the maximum observed. In the peroxidase test, the flower's ethyl acetate extract demonstrated the highest activity at 0.07 ± 0.008 Mm min -1 g -1DW.

Sisymbrium irio:

Antioxidant activity is abundant in the stem of Sisymbrium irio. In the stem lipid peroxidation assay, the DPPH assay revealed an IC50 value of 407.11 ± 18.09µg/ml. The FRAP assay confirmed that the stem of S. irio possesses the highest antioxidant potential of all its parts. In the peroxidase assay, the methanolic extract of the leaf of S. irio displayed the highest peroxidase activity, with a value of 0.028 ± 0.011 mMmin-1 g -1DW.

Digera muricata:

The methanolic extract from the stem of D. muricata had the highest radical scavenging percentage of 89.01 ± 1.23% when tested for antioxidant ability using the DPPH Assay.

Gomphrena celosioides Mart.:

The DPPH radical scavenging activity was reported to be maximum in the stem with a value of 88.04 ± 1.11% .

Trichosanthes cucumerina:

The petroleum ether extract of the leaf shows maximum DPPH radical scavenging activity with a value of 50.49 ± 0.84%. Methanolic extract of the plant showed maximum antioxidant capacity .

Melothria maderaspatana:

The DPPH radical scavenging activity was reported to be maximum in leaf with a value of 40.61 ± 0.41%. Among all the plant parts leaf shows maximum antioxidant activity .

Gomphrena celosioides:

The stem part was found to show maximum activity against E. coli may be due to the presence of phytosterols. Various extracts show potential activity against A. flavus, F. oxysporum. The methanolic extract shoes the maximum activity against A. flavus and A. fumigatus with an inhibition zone of 17 mm and 18mm .

Petrea volubilis:

Root shows antimicrobial capacity among all part parts it has activity against fungus P. funicolosum DIZ 16 mm MIC 12.5 mg/ml while the leaf and root against bacteria D. subtilis DIZ 25 mm MIC 3.13 mg/ml and against S. aureus DIZ 16 mm.

Melothria maderaspatana:

The petroleum ether extracts of leaf and fruit of M. maderaspatana shows the highest activity against T. reesai, and F. oxysporum DIZ 16 mm and the fruit has maximum potential against S. aureus and E. coli DIZ 10 mm, the ethyl acetate extract activity was maximum against P. funiculosum.

Trichosanthes cucumerina:

The fruit part shows Maximum activity against P. funiculosum and S. aureus DIZ 16 mm at 50 mg/ml. The leaf extract shows that it has moderate activity against bacteria such as P. aeruginosa, S. aureus, E. coli and B. subtilis .

Limonia acidissima:

The fruit component showed efficacy against T. ressei DIZ 14 mm, while the leaf part showed high and equivalent inhibitory activity against S. aureus and B. subtilis in the antibacterial assay. Mycobacterium tuberculosis was shown to be sensitive to methanol extracts of leaf GU-9, but resistant to fruit extracts, according to BACTEC TM MicroMGIT TM Assays.

Murraya koenigii:

In terms of effectiveness against bacteria and fungus, the leaf extract showed the best results, with MICs of 25 mg/ml and 18 mm, respectively, against E. coli and T. ressei. The antifungal activity of the leaf extract was found to be at its peak at 50 mg/ml when tested against P. funiculosum DIZ 14 mm MIC 25 mg/ml.

Anti-diabetic Activity:

Diabetes mellitus (DM) is a metabolic condition that disrupts carbohydrate metabolism due to an absolute or relative absence of insulin secretion. It is defined by persistent hyperglycemia, or an elevated blood glucose level. This condition is becoming more common over the world and is projected to affect 300 million people by the year 2025. The highest number of diabetic patients is anticipated to occur in India. Diabetes mellitus type 1 (insulin dependent) and type 2 (insulin independent) are the two main types of the disease. Insufficient insulin production by malfunctioning beta cells leads to type I diabetes. Diabetes mellitus type 1 (T1DM) patients must have insulin from an external source in order to control their blood sugar levels, while type 2 (insulin-independent) people can manage their condition with lifestyle modifications [31]. The non-insulin dependent diabetes mellitus type, which is characterized by high postprandial hyperglycemia, is the most common type of diabetes worldwide and in India.

The first step in hydrolyzing starch to maltose and then to glucose is catalyzed by pancreatic alpha-amylase, an essential digestive enzyme. Increased postprandial hyperglycemia (PPHG) results from the quick breakdown of this food starch. An important part of treating diabetes is keeping postprandial glucose levels under control, which has been linked to increased activity of human pancreatic amylase in the small intestine [32]. Therefore, a crucial role in diabetes control would be to postpone carbohydrate digestion by inhibiting enzymes like alpha-amylase. In the treatment of postprandial hyperglycemia, alpha-amylase inhibitors are crucial. Postprandial hyperglycemia is reduced because it inhibits the alpha-amylase enzyme, which reduces starch hydrolysis to maltose. Various glycosidases, including beta glycosidase and alpha-amylase, are known to be inhibited by the inhibitors acarbose, miglitol, and voglibose, which are presently used in clinical practice. Type II diabetes drugs such as biguanides, sulphonylureas, and thiazolidinediones are also on the market, but they come with their fair share of unwanted side effects [33–37]. Thus, research into hypoglycemic agents that are less harmful, more targeted, and more successful has persisted as a priority. Traditional medicinal plant extracts, which are naturally occurring, hold a lot of promise as a source for novel anti-diabetic medications.

T. cucumerina:

Alpha-amylase inhibitory activity of several T. cucumerina fruit extracts ranged from 25.23 ± 0.38% to 36.74 ±.59% in methanol, 40.47 ± 0.66% to 63.74 ± 1.14% in flavonoids, and 43.49 ±.72% to 52.22 ± 0.48% in alkaloids. The concentrations used were 0.3 to 1.5 mg/ml in each. The relative IC50 values of the following extracts: methanol (209.36 µg/ml), flavonoid (40.77 µg/ml), steroids (74.61 µg/ml), and alkaloids (135.5 µg/ml). The anti-diabetic potential of the plant is demonstrated by the considerable α-amylase activity found in its fruit and leaves, which are rich in flavonoids.

Melothria maderaspatana:

Percent inhibition activity of alpha-amylase by steroids and Alkaloids sample of the leaf of Melothria maderaspatana was evaluated. The IC50 value was calculated in the studies to be 65.10 µg/ml and 74.12 µg/ml. Steroids extracted shows significant antidiabetic potential from stem and flavonoids extracted from the fruit and leaf showed the highest inhibitory activity IC50 value was reported to be 61.42 µg/ml.

Aloe vera L.:

Various leaf extracts from Aloe vera L. were tested for their ability to inhibit alpha-amylase activity. The results showed that flavonoids and alkaloids exhibited inhibitory activity, with inhibition rates ranging from 55.83 ± 0.12 to 57.70 ± 0.09 percent and 7.36 ± 0.10 to 17.34 ± 0.10 percent, respectively, with an IC50 value of 0.0002 mg/ml and 0.032 mg/ml.

Azadirachta indica A Juss.:

Azadirachta indica A. Juss. leaf extracts containing flavonoids and alkaloids suppress alpha-amylase activity. At a concentration of 0.3 mg/ml to 1.5 mg/ml, 42.06 ± 0.3 to 46.85 ± 0.13 and 15.60 ± 0.11 to 19.69 ± 0.09 were observed, respectively. Flavonoids had an IC50 value of 0.009 mg/ml, while [38-43] alkaloids had an IC50 value of 16.66 mg/ml. The results showed that the plant's alkaloids had less antidiabetic action while flavonoids had more antidiabetic potential.

Allium cepa L.:

Flavonoids and alkaloids extracts of bulbs of Allium cepa L. showed 45.12 ± 0.16% to 47.83 ± 0.11% inhibition of alphaamylase at concentration of 0.3 to 1.5 mg/ml with an IC50 value of 5.37 mg/ml and 10.12 ± 0.11% to 16.37 ± 0.10% inhibition of alpha-amylase with 346.73 mg/ml respectively .

Allium sativum L.:

Alpha-amylase inhibitory activity of flavonoids and alkaloids extracts of bulbs of Allium sativum L. showed 56.22 ± 0.20% to 58.33 ± 0.20% with an IC50 value of 0.15 mg/ml and 10.22 ± 0.06 to 20.12 ± 0.10 with an IC50 value of 85.11 mg/ml respectively.

Mangifera indica L.:

Flavonoids and alkaloids of stem bark of Mangifera indica L. showed 55.15 ± 0.14% to 55.5 ± 0.14% with an IC50 value of and 1.29 ± 0.10% to 17.86 ± 0.13% with an IC50 value of 0.021 mg/ml and 3.168 mg/ml respectively.

Andrographis paniculata Nees:

Flavonoids of the whole plant, leaf, and root of the plant were found to have goof alpha-amylase inhibitory activity with an IC50 value of 0.2 mg/ml, 0.004 mg/ml and 0.017 mg/ml respectively .

Conclusion:

Secondary metabolites are an intriguing collection of bioactive chemicals that exhibit a wide range of activities against human cells, bacteria, fungus, viruses, and parasites, according to evolutionary pharmacology. The in-vitro methods for assessing secondary metabolites' antioxidant, antidiabetic, and antibacterial activities are the main topic of this review study. A thorough literature search was used to prepare it. The biological action of flavonoids, alkaloids, and steroids derived from several plants has recently been discovered. Anyone interested in the antioxidant, antidiabetic, and antibacterial properties of secondary metabolites will find this page to be an exhaustive and ready resource.

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