

Sustainable Phytocosmetics For Skin Brightening: A Review Of Natural Skin Brightening Agents With Emphasis Research Gap On *Symplocos Racemosa* And *Vetiveria Zizanioides*

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ABSTRACT

Skin hyperpigmentation is a common dermatological concern characterized by excessive melanin synthesis and uneven skin tone resulting from ultraviolet radiation, oxidative stress, inflammation, and hormonal imbalance. Conventional depigmenting agents such as hydroquinone and synthetic corticosteroids, though effective, are associated with adverse effects including irritation, ochronosis, and long-term cytotoxicity, prompting the demand for safer and sustainable alternatives. In recent years, phytocosmetics have emerged as a promising approach for skin brightening by utilizing plant-derived bioactive compounds with antioxidant, anti-inflammatory, and tyrosinase-inhibitory properties. Numerous botanicals such as Aloe vera, Glycyrrhiza glabra, Curcuma longa, and Azadirachta indica have demonstrated melanin-modulating potential through multi-targeted mechanisms. Among the underexplored medicinal plants, *Symplocos racemosa* Roxb. and *Vetiveria zizanioides* (syn. *Chrysopogon zizanioides*) possess significant traditional dermatological relevance and documented antioxidant and anti-inflammatory activities that may contribute to melanogenesis regulation. However, despite their ethnopharmacological importance, limited systematic studies have evaluated their direct tyrosinase inhibition, formulation compatibility, and in-vitro depigmenting efficacy in standardized cosmetic preparations. The present review critically summarizes natural skin-brightening agents, sustainable formulation strategies, and mechanistic insights into melanogenesis modulation, while identifying key research gaps related to phytochemical standardization, bioavailability, and clinical validation of *Symplocos racemosa* and *Vetiveria zizanioides*. Addressing these gaps may facilitate the development of safe, eco-friendly, and evidence-based herbal skin-brightening formulations aligned with green cosmeceutical principles..

Keywords: Sustainable phytocosmetics, Skin brightening agents, Hyperpigmentation, Tyrosinase inhibition, Melanogenesis, *Symplocos racemosa*, *Vetiveria zizanioides*, Natural bleach cream, Research gap..

INTRODUCTION

Hyperpigmentation becomes one of the aesthetic problems among humans including the male and female population in which darkened patches or spots appear on the skin. This may probably occur due to an overproduction and accumulation of melanin pigments or an increased amount of melanocytes expressing melanin synthesis within the skin layers [1,2]. Post inflammatory hyperpigmentation, solar lentigo, melasma, freckles and age spots are examples of hyperpigmentation problems. Melanin is a skin pigment that gives color to the skin, hair and eyes [3]. The variation in skin color among various races is

mainly determined by size and amount of melanosomes and the number, types and distribution of melanin in the suprabasal skin layer. Besides determining human skin color, melanin also plays a crucial protective role in human skin against the deleterious impacts of ultraviolet (UV) radiation, drugs, chemical substances and other environmental factors. As depicted in Figure 1, epidermal melanin units are arranged within the epidermis which consists of melanocytes surrounded by keratinocytes [4]. The presence of melanin units is very important for the melanin production. Melanogenesis or melanin biosynthesis is a complex sequential pathway that occurs in the melanosomes, membrane-bound

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organelles in melanocytes and catalyzed by tyrosinase as a rate limiting enzyme [5]. However, melanin production can be affected by various internal factors such as hormone regulation and inflammation as well as external factors such as UV exposure and drugs [1]. Consequently, this will lead to increased melanin production and hyperpigmentation. Therefore, cosmetic products containing skin lightening agents are considered as an effective approach towards this problem. Currently most cosmetic products contain

various types of skin lightening ingredients either found in natural, semi-synthetic or synthetic form [6]. In addition to resulting in a brighter and even complexion skin tone, it is claimed that skin lightening agents are used to prevent hyperpigmentation by reducing melanin production [2]. Different skin lightening agents possess distinct mechanisms of action on the melanin biosynthesis pathway.

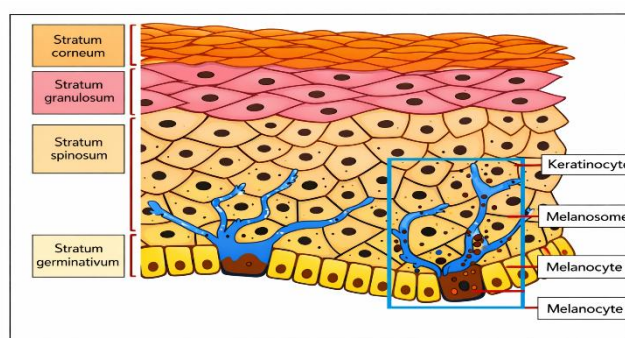


Figure 1: Layers of epidermis and an epidermal melanin unit (Represented by a blue box) [4]

However, the use of available skin lightening products commercialized in a market has some controversial and doubtful safety issues to the users. This is due to the incorporation of harmful whitening chemicals in the cosmetic products such as hydroquinone [7] and mercury [8] for an instant and greater lightening effect. It was reported that these chemical substances have serious safety concern among consumers as they exert many adverse effects to the skin and body health such as skin irritation, contact dermatitis, depigmentation, ochronosis [9] and organ toxicity [8]. As a result, the use of these toxic substances as skin lightening agents in cosmetic products is strictly banned by the Ministry of Health Malaysia to ensure consumer safety. Therefore, natural origin skin lightening products are preferable alternative over those chemical substances due to safely and cost effective and fewer side effects [10]. Natural skin lightening agents refers to biologically active constituents that are extracted and derived from natural sources such as plants, animals and minerals [11]. At the present time, many research studies have found various plant extracts known to have skin lightening activity due to the presence of particular bioactive compounds [12]. Hence, this review

highlights several natural skin lightening agents and their mechanisms of action in altering melanin biosynthesis pathway. In addition, some potential plants having skin lightening effects and their bioactive compounds that are responsible for lightening activity are also highlighted in this review. Mechanisms of action of natural skin lightening agents Currently, most cosmetic products contain various kinds of skin lightening ingredients which are extracted from natural sources of plant materials as well as microorganisms. The natural skin lightening ingredients such as arbutin, licorice and niacinamide are capable of preventing hyperpigmentation without initiating other skin problems [1]. Therefore, the incorporation of natural ingredients into cosmetic formulations is often preferable over synthetic substances. Generally, the induction of pigmentation can be controlled through many ways such as the inhibition of tyrosinase activity, inhibition of melanosome transfer, acceleration of epidermal turnover as well as the effect of antioxidant and anti-inflammatory agents [13]. However, among all targets, tyrosinase inhibition is one of the most important targets in minimizing melanin production and it is considered as the best approach in preventing

hyperpigmentation ^[14]. There are many processes involved in melanin biosynthesis in which different

skin lightening agents act at various targets and levels of the pigmentation process as illustrated in Figure 2.

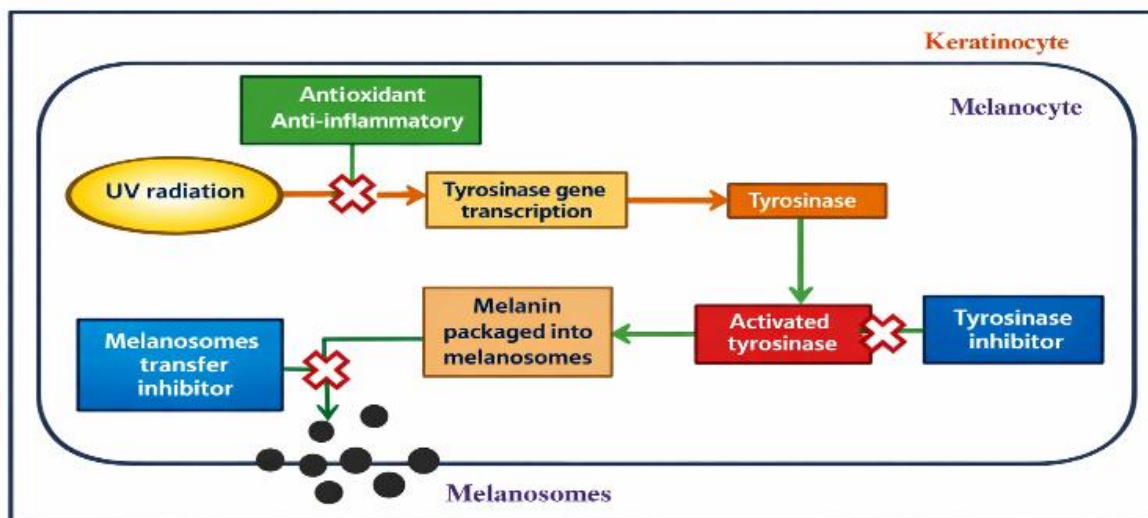


Figure 2: Mechanisms of action of skin lightening agents on melanin biosynthesis.

1.1 TRADITIONAL MEDICINAL SYSTEM

The complementary or traditional medicinal system among various cultural and socio-religious practices is significantly influences healthcare across the Indian subcontinent. Complementary and Alternative Medicine (CAM) in dermatology encompasses a diverse array of diagnostic and therapeutic approaches that enhance traditional dermatological procedures by integrating CAM principles with contemporary research insights.^[15] Complementary and Alternative Medicine (CAM) primarily emphasises holistic treatment of the human body with the expectation that this methodology will enhance dermatological care. The World Health Organisation (WHO) reports that over 80% of the global population depends on traditional herbal remedies. Approximately 40,000 to 70,000 kinds of medicinal plants are employed globally as traditional remedies. The global commerce in medicinal plants and their derivatives is currently valued at US\$ 100 billion with an annual growth rate of 15%.

India is one of the seventeen global mega biodiversity centres and encompassing four biodiversity hotspots: Western Ghats-Sri Lanka, Indo-Burma, Sundaland, and Himalaya (Figure 3).^[16] The Botanical Survey of India (BSI) has reported that among the 45,000 identified plant species at least 30,000 (two-thirds)

has significant therapeutic potential. Local inhabitants employ some plants as herbal remedies which are efficacious against various ailments. The utilisation of traditional remedies for dermatological conditions is a well-established and often employed practice in India. Contemporary healthcare institutions are frequently absent in remote regions. Consequently, conventional drugs are generally utilised to resolve healthcare concerns.^[17] A total of 84 kinds of medicinal plants are utilised in dermatology across India. Particular plant species were identified in four Indian states: Tamil Nadu (24 species), followed by Karnataka (17 species), and both Assam and Uttar Pradesh (nine species each) (Figure 3). The accessibility of herbal plants their reduced cost, and minimal or nonexistent adverse effects have led to an increasing demand for their use as therapies for numerous dermatological conditions.^[18] In India, despite the availability of modern medical systems, a significant segment of the population continues to pursue the advantages of various alternative therapies, such as Ayurvedic and Unani systems.



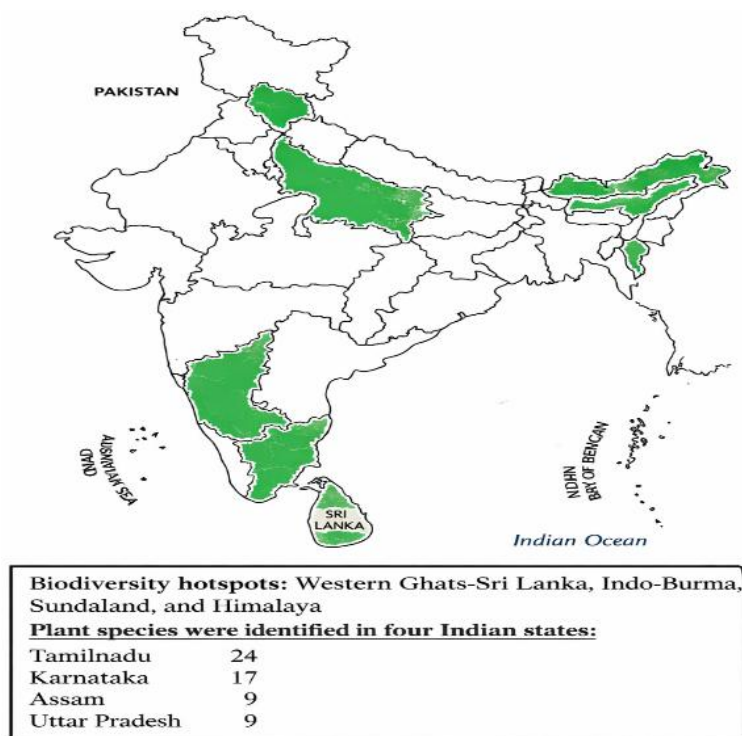


Figure 3: Number of plant species reported from specific Indian states

1.2 The Growth of the Asian Cosmetics Market and Rising Demand for Skin-Whitening Products

The Asian cosmetics market has experienced remarkable expansion over the past decade, positioning itself immediately behind Europe and the United States in global consumption. Major markets such as China, Japan, India, and South Korea have emerged as dominant contributors to this growth. In particular, China has demonstrated substantial retail performance, with total cosmetic sales exceeding USD 40 billion in recent years and maintaining a consistent upward growth trajectory, as reported by the National Bureau of Statistics (NBS, 2018). These figures reflect the increasing purchasing power, urbanization, and heightened consumer awareness regarding personal care and aesthetic enhancement across Asia.^[19]

Within this expanding market, skin-whitening and brightening products constitute a significant segment. Cultural perceptions associating lighter skin tone with beauty, youthfulness, and social prestige have driven sustained demand for depigmenting formulations. As a result, whitening creams, serums, masks, and lotions represent one of the most commercially successful categories in Asian cosmetics.

Concurrently, consumer preference is shifting toward safer and more natural alternatives due to growing concerns about the adverse effects associated with synthetic bleaching agents. Natural products are widely perceived as skin-friendly, biocompatible, and environmentally sustainable. Consequently, herbal and plant-based whitening agents have gained substantial acceptance among consumers.^[20] Botanical ingredients with antioxidant, anti-inflammatory, and tyrosinase-inhibitory properties are increasingly incorporated into modern cosmeceutical formulations.

1.3 The Effects and Problems of Traditional Asian Herbs in Skin Whitening

The application of herbs in skin whitening starts quite early in Asia. The book *Shen Nong's Herbal Classic* written in more than 2,000 years suggested that semen platycladi, the seed kernel of *Platycladus orientalis* (L.) Franco can improve people's complexion and appearance. The book *Theory of Medicine Nature* recorded that the rhizome of *Atractylodes macrocephala* Koidz. can ameliorate dark skin^[21]. After thousands of years' attempts in developing whitening reagents, lots of useful Asian traditional herbs have been recorded, and some of

them have been studied in recent years [22]. Generally, how traditional herbs are used can be divided into three types: formula (consisted of several herbs); extract (a mixture of several components, or a class of compounds, from the same herb), and active ingredient (a purified compound that has a definite molecular structure).

1.4 Herbal Formulas

Herbal formulas function through the synergistic interaction of multiple components, where each herb plays a specific role, and the overall composition follows defined principles to enhance therapeutic efficacy while minimizing adverse effects [23]. Herbal formulas account for a significant proportion of traditional herbal usage; however, studies focusing on formula-based approaches for skin whitening remain limited. In one study, researchers screened 50 herbal preparations (32 single herbs and 18 herbal formulas) and successfully identified three effective tyrosinase inhibitors [24]. Further investigation revealed that Qian-wang-hong-bai-san inhibited the p38 MAPK and PKA signaling pathways, whereas San-bai-tang suppressed the p38 MAPK signaling pathway [24]. Although these formulas have existed for a long time, this was the first study to elucidate their underlying molecular mechanisms.

In addition, a Thai herbal formula, AVS073, was reported to regulate melanogenesis by suppressing tyrosinase activity and reducing oxidative stress through enhancement of glutathione (GSH) biosynthesis and glutathione S-transferase (GST) activity [25]. A Korean herbal formula, LASAP-C, demonstrated anti-melanogenic efficacy by downregulating melanogenic proteins, including tyrosinase (TYR), tyrosinase-related protein 1 (TYRP1), and tyrosinase-related protein 2 (TYRP2), both in cellular models and zebrafish systems [26].

Moreover, India possesses a long-standing tradition of herbal formulations under the ancient medical system of Ayurveda. Consequently, several Indian herbal preparations have been evaluated in recent years. Ubtan, a traditional Ayurvedic formulation, has been reported to exhibit significant anti-tyrosinase and antioxidant activities [27]. (The composition of formulas showed in [Table 1.](#))

Study	Formula	Species / Composition (Ratio / Concentration)	Mechanisms	Models
Ye et al. (2010a); Tsang et al. (2012)	Qian-wang-hong-bai-san	Tubers of <i>Bletilla striata</i> (Thunb.) Reichb. f.; tubers of <i>Sauromatum giganteum</i> (Engl.) Cusimano & Hett. (syn. <i>Typhonium giganteum</i> Engl.); pericarps of <i>Punica granatum</i> L.; fruits of <i>Benincasa hispida</i> (Thunb.) Cogn.; ratio 1:1:1:1	Suppressed tyrosinase activity and expression; inhibited p38 MAPK signaling pathway; inhibited PKA/CREB signaling pathway	Mushroom tyrosinase; B16 cells
Ye et al. (2010a)	Qiong-yu-gao	Roots and rhizomes of <i>Panax ginseng</i> C.A. Mey.; roots of <i>Rehmannia glutinosa</i> Libosch.; rhizomes of <i>Smilax glabra</i> Roxb.; ratio 1:1:1	Suppressed tyrosinase activity	Mushroom tyrosinase; B16 cells
Ye et al. (2010a); Ye et al. (2010b)	San-bai-tang	Rhizomes of <i>Atractylodes macrocephala</i> Koidz.; rhizomes of <i>Smilax glabra</i> Roxb.; roots of <i>Paeonia lactiflora</i> Pall.; ratio 1:1:1	Suppressed tyrosinase activity and expression; inhibited p38 MAPK signaling pathway and MITF expression	Mushroom tyrosinase; B16 cells

Panich et al. (2013)	Ayurved Siriraj Brand Wattana formula (AVS073)	<i>Piper nigrum</i> (L.); <i>Boesenbergia rotunda</i> (L.) Manf.; <i>Cyperus rotundus</i> (L.); <i>Tinospora crispa</i> (L.) Hook.f. & Thomson; <i>Terminalia chebula</i> Retz.; <i>Cladogynos orientalis</i> Zipp. ex Span.; <i>Derris scandens</i> (Roxb.) Benth.; <i>Anamirta cocculus</i> (L.) Wight & Arn.; <i>Putranjiva roxburghii</i> Wall.; <i>Cinnamomum siamense</i> Craib.; <i>Ferula assa-foetida</i> L.; <i>Aegle marmelos</i> (L.) Corrêa; <i>Conioselinum vaginatum</i> (Spreng.) Thell.; <i>Aucklandia costus</i> Falc.; <i>Cryptolepis dubia</i> (Burm.f.) M.R.Almeida; concentration not provided	Suppressed tyrosinase activity and mRNA; inhibited ROS formation	G361 cells
Kim et al. (2016)	LASAP-C	Root of <i>Rehmannia glutinosa</i> Libosch. var. <i>purpurea</i> Makino; 100 g fruit of <i>Lycium chinense</i> Mill.; 50 g root of <i>Scutellaria baicalensis</i> Georgi; 50 g root of <i>Angelica dahurica</i> (Hoffm.) Benth. & Hook.f. ex Franch. & Sav.; 35 g	Suppressed tyrosinase activity and expression	B16F10 cells; zebrafish

Table 1: Herbal Formulas with Anti-Melanogenic Activity

1.4 Relevance to Selected Herbs *Symplocos racemosa* and *Vetiveria zizanioides*

tyrosinase-inhibitory mentions support its potential role in melanogenesis modulation. The reported Skin brightening studies are mentioned in table 2

Direct studies on *Symplocos racemosa* for skin brightening are limited; however, antioxidant and

Plant species	Study (Author, Year)	Extract / Test Material	Mechanism(s) Reported	Model / Activity
<i>Vetiveria zizanioides</i>	Peng et al. (2014)	Vetiver essential oil (VZ-EO)	↓ Melanin production; ↓ Tyrosinase expression; Antioxidant action (↓ oxidants & MDA, ↑ SOD, GPX, CAT)	α-MSH stimulated B16 melanoma cells – hypopigmentation effect observed
<i>Symplocos racemosa</i>	(Traditional & review mentions)	Bark extract / Phytochemicals	Reported antioxidant and mild tyrosinase inhibition potential (≈6.60% inhibition) supporting skin care use	Ethnobotanical review (tyrosinase potential implied)
<i>Symplocos racemosa</i>	Gowthami & Poda (2025)	Bark extract (phytochemistry & antioxidant)	Significant antioxidant activity (DPPH, ABTS), which is associated with melanogenesis modulation pathways	In vitro antioxidant assays (linked to pigment modulation potential)

Table 2: Relevance to Selected Herbs *Symplocos racemosa* and *Vetiveria zizanioides*

1. **Vetiveria zizanioides essential oil** has been experimentally shown to reduce melanogenesis by suppressing tyrosinase expression and oxidative stress pathways in melanoma cells, indicating hypopigmenting potential relevant to cosmeceutical applications.
2. **Symplocos racemosa** does not yet have direct studies explicitly examining skin-brightening or tyrosinase inhibition in melanocyte models in indexed journals; however, reviews on Ayurvedic herbs report **mild tyrosinase inhibition activity** and traditional skin care uses that support its inclusion in natural pigment-modulating formulations.
3. Recent phytochemical and antioxidant studies on *Symplocos racemosa* extract show **robust antioxidant activity**, which is closely associated with reduced oxidative stress-mediated melanogenesis inhibition and supports its potential role in depigmenting formulations.

2.0 METHODOLOGY

Various key phrases, including “Indian traditional ethnodermatology,” “ethnodermatology,” “ethnobotany,” “skin diseases,” “Ayurveda dermatology,” and “pharmacological activity,” were utilized in online search engines and databases such as Google Scholar (<https://scholar.google.com/>), ResearchGate (<https://www.researchgate.net/>), PubMed (<https://pubmed.ncbi.nlm.nih.gov/>), Scopus, and NISCAIR Online Periodicals Repository (NOPR) (<http://nopr.niscair.res.in/>), thereby employing the relevant databases and keywords. This study exclusively covered papers published in English from 2000 to October 2024 (Fig. 2). A total of 1078 studies were included following an initial screening of 453 published papers. Based on data acquired from several online search engines, we developed numerous significant discoveries. Table 1 delineates plant species utilized in traditional medicine for dermatological ailments in India; Table 2 elucidates the pharmacological properties of medicinal plants along with their preclinical evidence; Table 3 details clinical evidence regarding the use of plants in humans. Species nomenclature was verified using The Plant List 1.1 (“The Plant List,” 2013), while family classifications adhere to the Angiosperm Phylogeny Group IV.^[28]

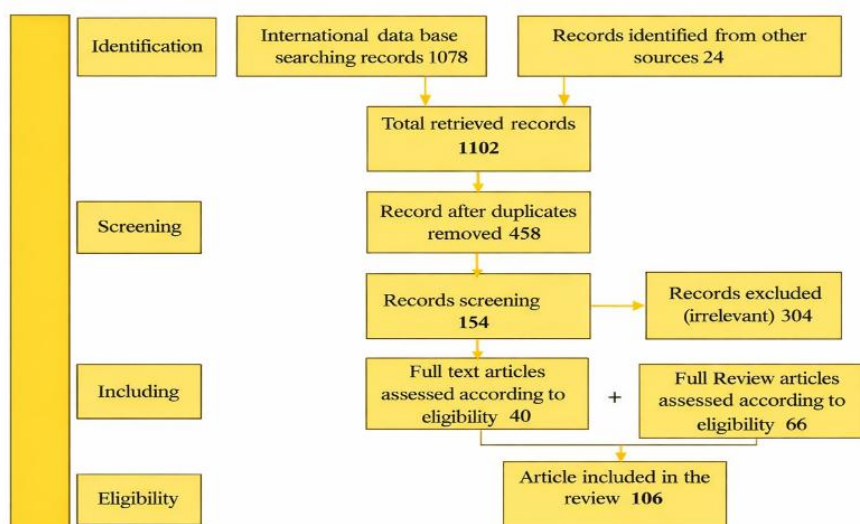


Figure 4: Screening research and review articles for the composition of review papers.

3.0 LITERATURE REVIEW

The literature review provides a systematic overview of previously published research relevant to the present study. It helps to understand the current state of knowledge, identify established findings, and recognize existing research gaps. In the context of skin brightening and herbal formulations, the literature highlights the mechanisms of

melanogenesis, the role of synthetic depigmenting agents, and the growing interest in plant-based alternatives. Previous studies on natural extracts, phytochemicals, and traditional formulations offer scientific evidence supporting their anti-melanogenic and antioxidant properties. Reviewing these studies provides a strong theoretical foundation for developing safe and effective herbal skin-brightening formulations

3.1 Botanical Extracts with Depigmenting Activity

Component	Plant Source	Mechanism	Model	Key Finding	Ref.
Arbutin	Pear, cranberry	↓ Tyrosinase, ↓ DHICA polymerase	MT, B16	Moderate depigmenting effect	[29]
α -Arbutin	Pear, cranberry	↓ Tyrosinase	MT (human melanocytes)	More potent than arbutin	[30]
Deoxyarbutin	Bearberry	↓ Tyrosinase	NHM	Improved skin tone	[31]
Aloesin	Aloe	Competitive ↓ Tyrosinase	B16	Reduced pigmentation	[32]
Flavonoids	Various plants	↓ Tyrosinase, copper chelation	MT	Anti-melanogenic activity	[33]
Hesperidin	Citrus fruits	↓ Tyrosinase, antioxidant	B16	Reduced UV-induced pigmentation	[34]
p-Coumaric acid	Panax ginseng	↓ L-tyrosine oxidation	B16	Suppressed melanogenesis	[35]
Niacinamide	Root vegetables, yeast	↓ Melanin transfer	Co-culture model	35–68% reduction in melanin transfer	[36]
Glabridin	Licorice	↓ Tyrosinase, ROS scavenger	B16	Significant inhibition at low dose	[37]
Liquiritin	Licorice	Epidermal dispersion	Human study	80% improvement in melasma	[38]
Mulberry extract	Morus alba	↓ Tyrosinase, antioxidant	Melan-a	Comparable to kojic acid	[39]
Procyanidins	Grape seed	↓ Tyrosinase, ROS scavenger	B16	Strong anti-melanogenic effect	[40]
Ellagic acid	Strawberry, geranium	Copper chelation	B16	Reduced melanin synthesis	[41]
18 β -GL	Licorice	↓ Tyrosinase	S91	Reduced UV-induced pigmentation	[42]
Sophorcarpidine	Kusheng	↓ Tyrosinase	Melan-a	Depigmenting effect	[43]

Table:2 Selected Botanical Extracts with Depigmenting Activity



Figure 1: Hyphenated technique

A new dimension in writing technology that offers many important advantages in pharmaceutical analysis is multivariate chromatography. Several parameters were investigated, including the combination of GC, HPLC and CE systems under different conditions to analyze different types of samples [3]. Examples include size exclusion chromatography and RP-HPLC, EC, GC and LC. Since RP-HPLC and CE techniques can provide high resolution separation using orthogonal separation mechanisms, combining the two methods in 2D provides very high peak performance and very high resolution, especially for complex blending [4]

Advantages of hyphenated techniques [5]

1. Fast and accurate analysis
2. High degree of automation
3. High throughput
4. Excellent reproducibility
5. Separation and quantification are performed simultaneously.
6. Provides superior separation and optional online spectroscopic data collection for LC or GC peaks of complex mixtures.

Types of Hyphenated Technique [6,7,8]:

GC-MS[2]:

When using MS as the recommended detection method and using one and three quadrupole mass spectrometers, ion attack and time of flight (TOF) are the most common instruments, while LC- MS and GC are the two most common methods. Today. GC / MS is a technology developed by linking GC and MS and is the first technology to be used for research and development purposes. The mass spectrum obtained with this hyphenation technique provides more structural information based on fragmentation analysis. GC / MS analysis may require polar compounds, especially those with many hydroxyl groups. The most common derivatization method is the conversion of the analyte to a trimethylsilyl derivative.

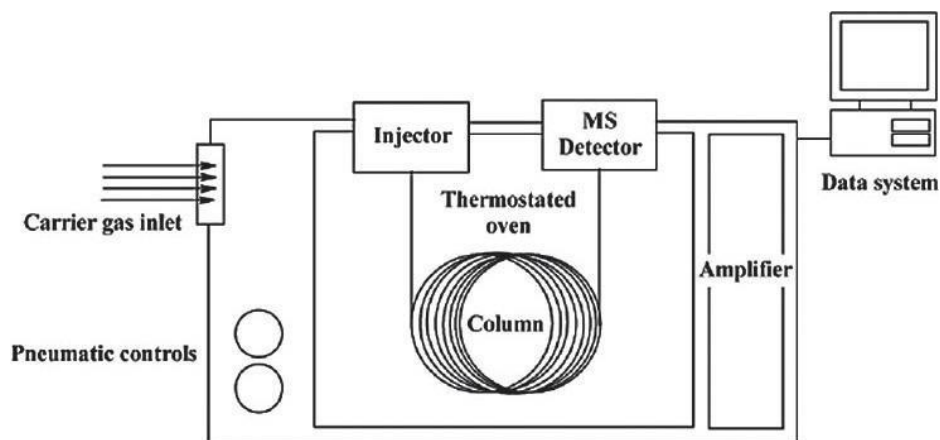


Figure.2: GC-MS

Instrumentation and Working:

When the analyte is vaporized by the heated carrier gas, it passes through the GC column, it is simply separated from the column. The operator is also called the mobile phase, helium. The identified interaction of the analyte between the mobile and stationary phases disrupts the relationship. The separation of analytes also depends on the column dimensions (length, diameter, film thickness), the type of carrier gas, the column temperature (gradient), and the characteristics of the stationary phase. The path of the sample through the column, differences in boiling points and other chemical separation factors of the mixture components. Due to differences in adsorption or separation between the mobile phase and the stationary phase, the elution and retention times of the components differ. The individual components of the mixture enter the MS via the interface. This is followed by ionization, mass spectrometry, and the determination of the mass-to-charge ratio of the ions produced by each analyte using mass spectrometry. Interfaces are available in the form of effect splitters, beam / aperture splitters, and diaphragm splitters to connect the GC to the MS. The ionization process not only ionizes molecules, but also breaks them down into fragments and detects these fragments through electron collisions and chemical ionization. The analyte molecular ions produce a spectrum of fingerprints that is different from that of other analytes. GC / MS is an essential tool in analytical chemistry. This technology is designed to accurately isolate, identify and provide information about the structure and composition of very small samples. The advantage of this method is that two different analytes can have the same mass spectrum, but since the retention times of the two analytes are different, GC-MS can be used to separate or analyze these types of analytes. Can you do this. Two ionization methods that are widely used in

GCMS are electron bombardment & the alternative chemical ionization (CI) in either positive or negative modes ^[1]

Applications [10]:

1. Quantification of drinking water and wastewater contamination according to official US Environmental Protection Agency (EPA) methods.
2. The quantitative determination of metabolites and drugs in urine is carried out for pharmacological and forensic purposes.
3. Identification of unknown organic compounds at hazardous landfills and in reaction products using synthetic organic chemistry.
4. Used in the analysis of drugs, pesticides and herbicides.

LC-MS :

LC-MS stands for a combination of LC and mass spectrometry (MS) [Figure 3]. Individual samples in a column can be identified based on mass spectral data. Changeover valves help functionally combine the two technologies. A typical automated LC-MS system consists of an autosampler, an LC system, and a three-way dual transducer and mass spectrometer. The transducer typically acts as an automatic shutoff valve to drain unwanted portions of the LC system eluate into the trash before the sample enters the MS. LC-MS combines the function of MS with chemical separation LC for selective detection, verification and confirmation of molecular identity. MS is one of the most sensitive and selective methods of molecular analysis, which provides information on the molecular weight and fragmentation pattern of the analyzed molecules. The information obtained by MS is very important in determining the molecular identity of an analyte

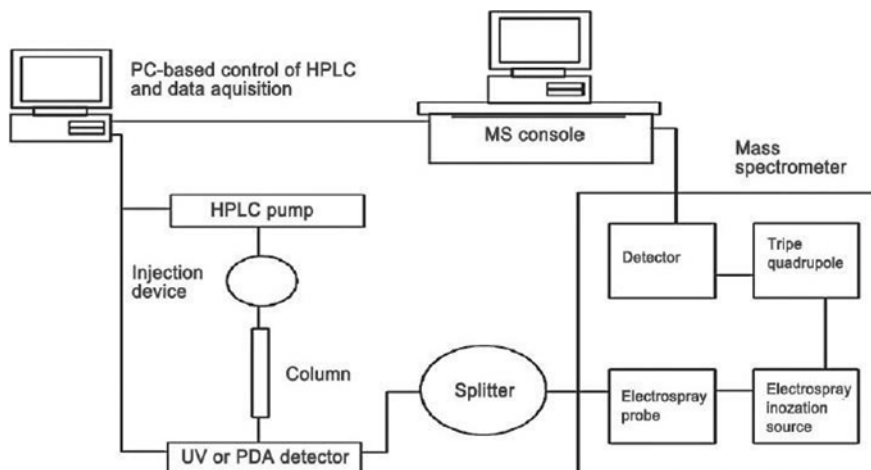


Figure 3:LC-MS

Instrumentation and working:

The LC-MS device can be associated with electrospray, particle beam, and thermal spray. Electrospray is the most commonly used interface. The syringe serves as a bridge between liquid chromatography and mass chromatography. However, independent radiators are flexible and practical. [9-12] Changeover valves help create a functional combination of the two technologies. A typical automated LC-MS system (Fig. 4) consists of a double three-way catalyst with the following characteristics:

1. Autosampler
2. LC system
3. Mass spectrometer.

The inverter typically acts as an automatic shutoff valve to divert unwanted portions of the eluate

from the LC system into contaminants before the sample enters the MS. The ionization method used in LCMS is a weak ionization method that usually shows only molecular ions and a small number of fragment ions. Thus, the information on the structure of the compound obtained using a single LC-MS method is very weak. However, this problem is currently being solved by introducing tandem mass spectrometry (MS-MS), which provides fragments with collisional dissociation of the resulting molecular ions. The use of LC-MS-MS is growing rapidly. LC-MS combines the function of MS with chemical separation LC for selective detection and confirmation of molecular identity. MS is one of the most sensitive and selective methods of molecular analysis, which provides information on the molecular weight and fragmentation pattern of the analyzed molecules. The information obtained using MS is very important for the identification of analyte molecules [13, 14, 15]

Applications:

1. LC-MS for the detection of compounds from polycyclic (non-polar) aromatic compounds to peptides and proteins.
2. LC-MS for identification and purity of compounds.
3. Used to measure pesticides, herbicides and organic pollutants for environmental monitoring.
4. Provides information on the molecular weight and fragmentation pattern of analyte molecules.
5. This technique was used for proteomic analysis.
[20]

LC-NMR[6,8] :

In this technology, technological development allows the HPLC system and NMR to be directly coupled in parallel, resulting in a new practical technology, HPLC-NMR or LCNMR, which has

been widely known for more than 15 years. The first online HPLC-NMR experiment using superconducting magnets was reported in the early 1980s. However, the use of this scripted technique in analytical laboratories did not begin until the late 1990s. In fact, the benefits of closed-loop identification and separation circuits, along with the prospect of using all currently available 2D and 3D NMR technologies in a fully automated manner, have promoted the development of stopped-flow modes, such as time-delay modes. A typical LC-NMR experimental device is shown in Figure 4. LC-NMR experiments can be performed in continuous flow mode and discontinuous flow mode. Using 500, 600 and 800 MHz systems with ^1H , ^{13}C , ^2H , ^{19}F and ^{31}P probes can solve a wide range of biological analysis problems. In addition to NMR and HPLC instruments, the main prerequisites for online LC-NMR are the flow-through probe and the valve installed before the probe to record flow-through or stopped-flow NMR spectra.

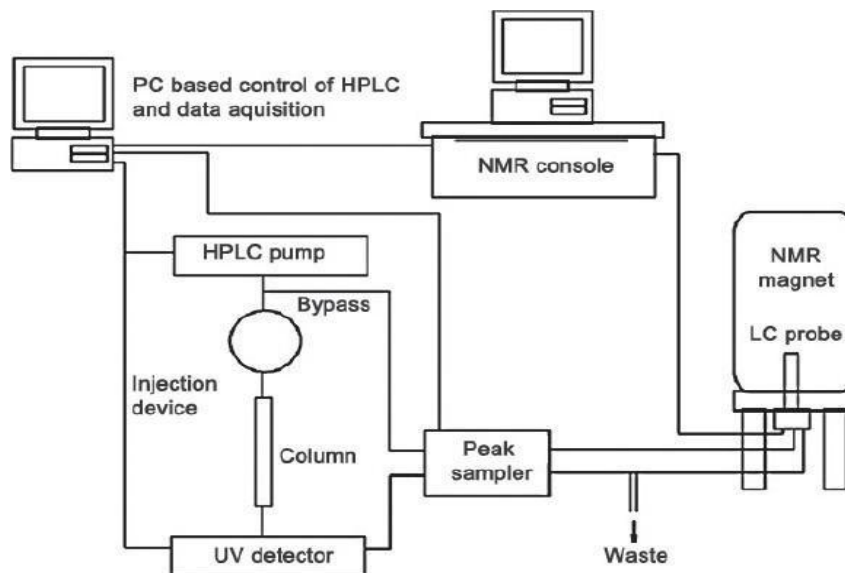


Figure 4: LC-NMR

Instrumentation and working:

LC-NMR is a sensitive technique because it uses a magnet with a strong magnetic field and a sensitive probe, and the NMR sensitivity can be increased by outdated technologies such as solvent removal techniques and suitable software for automated measurements. For multi-component analysis. The stronger the external magnetic field, the higher the sensitivity^[16]. The increased sensitivity significantly shortens the measurement time. Compounds with complex spectra can be easily resolved with a higher magnetic field, resulting in better signal resolution.^[17]

Applications:

1. Provides information describing the structure of natural products.
2. Analysis of all kinds of complex mixtures, especially natural substances and drug-related metabolites in biological fluids.

3. This technology is used to detect pesticides, herbicides and organic pollutants for environmental monitoring.

CE-MS:

CE is an automatic separation technology that was introduced in the early 1990s. FE analysis is performed under the action of an electric field in a thin tube that can quickly separate hundreds of different compounds. The flexibility and number of methods used for CE means that virtually any molecule can be isolated in this reliable way. It is commonly used to separate seeds by applying voltage to buffer-filled capillaries and to separate ions that move at different speeds depending on their size and charge when voltage is applied. A solution is considered a peak when it passes through the detector, and the area of each peak is proportional to its concentration, allowing for quantitative measurements^[21]. Testing includes purity measurement, test and trace measurement. When an EM detector is connected to a CE system to obtain live EM data for isolated connections, the resulting combination is called CE-MS [Figure 5].

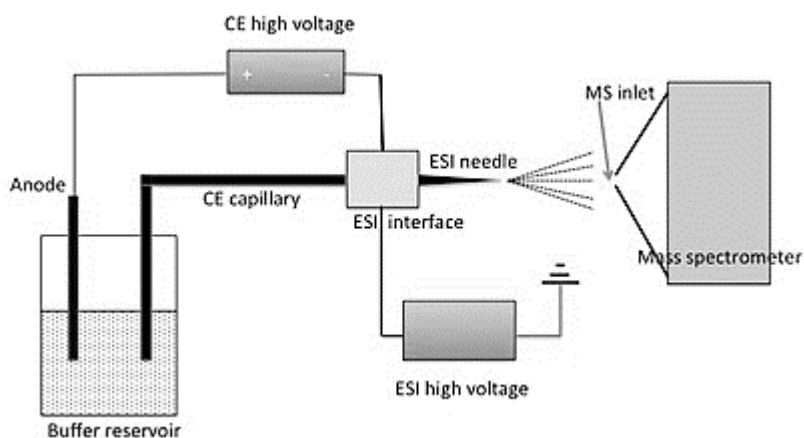


Figure 5: CE-MS

Instrumentation & working:

The detectors used for CE analysis are UV and DAD detectors, and the electrolytes used in this method are inorganic and non-volatile. This method has several disadvantages, including insufficient reproducibility, reproducibility and selectivity. This problem can be solved by using a buffer system that covers the inner walls of silica capillaries connected by a double layer. Very small volumetric streams are transmitted via the interface to the MS. The ionization interface used is electrospray. Fast atomic bomb interface and ion spray ionizer. Mass spectrometry uses TOF detectors, ion traps and quadrupoles. The biggest

advantage of a quadrupole detector is its sensitivity. [22]

Application

1. Bases and acids can be identified using non-aqueous CE-MS and non-aqueous CE-MS.
2. Analyze complex arabinooligosaccharides.
3. CE-MS is a drug and biomarker discovery tool.

Applications of Hyphenated Technique in Pharmacy [23,24,25,26]:

Figure 6 shows some examples of the application of methods for the analysis of natural materials

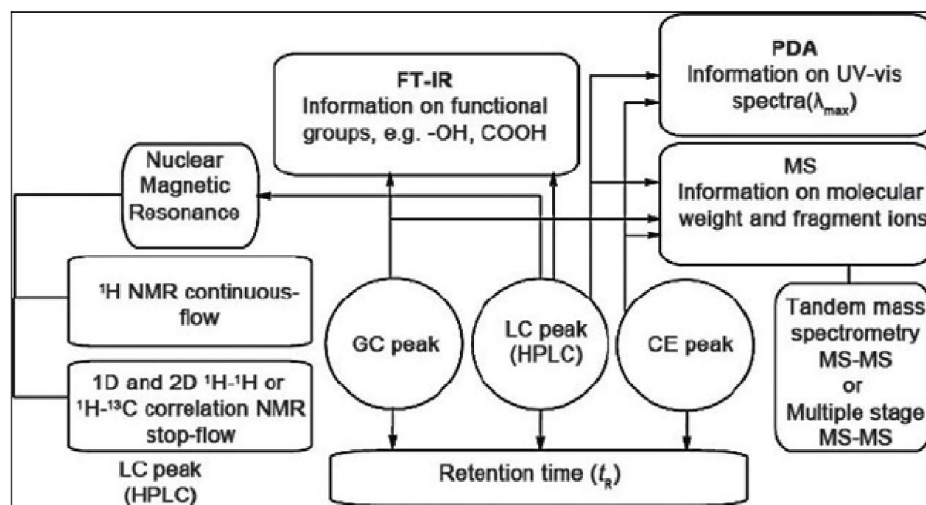


Figure 6. Applications of Hyphenated Techniques Isolation and analysis of natural products:

Isolation and analysis of natural products

Crude extracts of natural ingredients, which are very complex mixtures of many compounds, can be successfully analyzed using appropriate writing techniques. Among the various methods, LC-PDA and LCMS are the two most commonly used for the analysis of natural products. In addition to LC-NMR, various multiple binding techniques have

recently been developed such as LC-PDA-NMRMS. If the ionization method is chosen correctly, LC-MS can be a very powerful and informative tool for the selection of crude plant extracts. The various types of LC-MS systems currently available allow the analysis of small non-polar compounds for large polar components such as oligosaccharides, proteins and tannins, which are present in natural product extracts [18]

Alkaloids

Alkaloids are a large group of secondary metabolites of plant, microbial or animal origin. Contains nitrogen. So far, different methods have been used to analyze different types of alkaloids. With the development and increasing availability of benchtop systems, GC / MS has become the method of choice for the analysis of several types of pyrrolizidine and quinolidine alkaloids. The alkaloids of cynolididine, an important class of legume alkaloids, have recently been analyzed by GC-MS. [25] Most of these alkaloids are thermally stable under GC conditions, they are quite volatile and can be analyzed without chemical modification. However, some hydroxylated pyrolicidine alkaloids should be interpreted as trimethylsilyl derivatives. Ephedrine-type alkaloids in Chinese herbal medicine and nutritional supplements were analyzed by GC-MS and GC-FTIR methods.

Coumarins

Coumarins are the largest class 1 benzopyran derivatives, found primarily in higher plants. HPLC-PDA can be used to analyze a variety of phenolic compounds, including coumarins. This is due to the large number of chromophores present in these molecules. Measurement of coumarins with HPLC-PDA, in which the absorption spectrum is recorded on a PDA detector, provides useful information on molecular identity, including oxidation patterns. The retention time, together with the UV spectrum of each peak, can be considered a characteristic and can be easily used to detect known coumarins in crude extracts. The relationship between MS and LC-PDA provides additional structural information for online identification of individual coumarins in crude extracts. Several coumarins have been analyzed by atmospheric ionization along with other heterocyclic oxygen compounds found in the non-volatile residues of mandarin, sweet orange, bitter orange, bergamot, and grapefruit citrus oils (e.g. psoralen and polymethoxylated). LC-MS system with APCI probe in positive mode.^[19]

Carotenoids

Natural substances in this group include hydrocarbons (carotene) and their oxygen-rich derivatives (xanthophylls). LC-TLS has been

successfully used for the quantitative determination of carotenoids from four phyto- and diatoxanthin, diatoxanthin and other carotenoids are also separated with higher sensitivity and selectivity than UV detection achieved by isocratic HPLC elution. This method monitors the conversion of diatoxanthin to diatoxanthin and changes in other carotenoids under various lighting conditions^[27]. LC-TLS is also a very sensitive method for measuring carotene in fish based on complement oil.^[28]

Essential oil and volatile components

GC / MS has established itself as an analytical tool, especially for the analysis of non-polar and volatile natural materials such as mono- and sesquiterpenes^[29] described how the direct expansion of GC-MS can measure approximately 130 volatiles in various herbs. They used the efficient GC- MS method using EI to isolate and assemble the de-esterified components of Chinese herbal medicine essential oils, Jilin ginseng, root and orange peel. Pestasia Atlanticaba. The main component of the monoterpenes of essential oils is oleoresin. mutica was recently analyzed by GC/ MS in collaboration with an online database application.^[30]

Dereplication:

Differences between previously tested or processed natural product extracts can be demonstrated by reducing the mass collection of isolated isolates requiring more accurate assessment. Isolation of natural substances based on biological tests often results in compounds known to have limited or no chemical or pharmacological significance. Consequently, modern research on profitable natural product requires appropriate early differentiation methods for new natural products instead of known or already isolated natural products. The cloning strategy uses a combination of separation science, spectroscopic detection techniques, and online database searches. Thus, by combining HPLC with structurally advantageous spectroscopic detection methods (PDA, MS, NMR, etc.), extracts or crude fractions can be selected based on structural classification as well as biological activity. Biological testing and HPLC analysis using other detection methods were used to verify the



effectiveness of the extract. Technologies such as HPLC combined with UV- photodiode detection (LC-DAD-UV) and mass spectrometry (LC-MS or LC-MS-MS) provide rich on-line analytical data for pre-isolated extracts. Combined HPLC with NMR (LC-NMR) is a powerful addition to LCUV-MS screening.

Chemotaxonomy:

Chemical classification, or chemical classification, is based on the principle that the presence of certain secondary metabolites is determined by various enzymes involved in the biosynthesis of these compounds. Thus, chemical profiling of these secondary metabolites, either through complete isolation and identification, or online isolation and identification using modern hyphenation techniques, can link and provide useful information for taxonomic or phylogenetic relationships between different species.

Chemical Fingerprinting and Quality Control of Herbal Medicine [30,31] :

In recent years, much attention has been paid to the use of hyphenation techniques in chemical fingerprint analysis for quality control and standardization of herbs, such as LC-MS, CE-MS, LC- NMR or LCNMR-MS. The fingerprint technique is often used to isolate the matrix profile of a sample in the context of drug analysis. This is often enough to show the sauce and its preparation. For medicinal herbs, the profile depends not only on the production process, but also on the quality of the herbal raw materials. Conforms to renowned standards for assembly of structures. The integrated MS database also helps to identify these connections. Thus, GC-MS, LC-MS and MS- MS fingerprint profiles were obtained from the active ingredients of various herbal extracts, and the information was stored in the form of an electronic database that could be used for routine comparisons. Analysis of the chemical profile of selected plant extracts for quality control. GC or LC data retention times and mass spectral data can be reproduced as long as the chromatographic and spectroscopic conditions remain constant. LC-NMR and LC-NMRMS are also used for this purpose. A simple protocol for chemical fingerprinting of ephedra using HPLC-PDA has recently been described. [33] Ephedra

sinica (Ephedra family), also known as ‘Ma huang’, is one of the oldest medicinal plants used in traditional herbal medicine. In Europe and the United States, E. with Sinica food supplement has become one of the top-selling weight loss and stamina products, used by over a million consumers.

Metabolomics:

The term “metabolomics” refers to the complete replacement of low molecular weight metabolites in living cells and is also used to describe the observed chemical profile or fingerprint of metabolites in tissues.^[34] Metabolites reflect the life history of an individual organism, including environmental factors such as age, soil type, moisture content, temperature, and stressors. Research involving detailed analysis of these metabolites is known as “metabolomics” and is a new area of research on natural products in the post-genomic era. The goal of metabolomics, like plant metabolomics, is to provide a better understanding of metabolism or other physiological phenotypes through global genome-related technologies. The profiling of off-target metabolites and the linking of these profiles to genotype or transcriptional profiles in metabolomics is considered an important topic..^[35]

CONCLUSION:

The technology developed by combining separation technology and online spectroscopic detection technology is called combined technology. The tremendous progress in written analytical methods over the past two decades has greatly expanded their application to the analysis of biomaterials, especially natural products. This article was recently developed using various methods such as GC-MS, LC-MS, LC-NMR, and CE-MS. Preliminary analysis of raw extracts or fractions from various natural sources, online isolation and detection of natural products, chemical taxonomic studies, chemical fingerprint analysis, quality control of plant products, deduplication of natural products and related contexts are discussed. For example, research on metabolomics. Emphasis is placed on written techniques, including LC as a cutting tool.



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