Isolation and Structure Elucidation of Natural Products Chemistry – Review of Modern Trends (Alkaloids and Terpenes)

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Abstract: Natural products are also known as secondary metabolites, compounds that are naturally produced by biological organisms. These natural products have a broad range of functions which include agents that solubilize and transport nutrients, like siderophores which transport iron Fe^{3+} across membranes in bacteria and fungi; they are often structurally complex molecules that possess a well-defined spatial orientation. These compounds have evolved to interact efficiently with their biological targets, as their producers evolved alongside their target organisms. This work aimed at reviewing modern trends in isolation and structural elucidation of Alkaloids and Terpenes. Alkaloids are the main secondary metabolites which have medical properties. It can be used to avoid the several degenerative sicknesses by scavenging free radical or binding with catalyst, the oxidative reaction. Terpenes or terpenoids are the major diverse group occurring in plants naturally, depending on isoprene unit's number. Humans used several of terpenoid compounds to produce important compounds such as vitamin A from β-carotene. Generally, terpenes in foods contain a main effect on our eating practice. Terpenoid as pigments, like bixin, astaxanthin, and lycopene are utilized in the food manufacturing industry. The modern techniques as well as the advanced instruments that employed in the discovery programme of bioactive natural products facilitated the task of the natural product chemists and permitted them to detect, target, isolate and elucidate the structure of the pharmacologically active natural product in significantly short times compared with that done in the not too distant past. Conclusively, the review identified the various modern methods that are in use in the isolation, purification and structure elucidation of alkaloids and terpenoids. these include NMR,MS and hyphenated HPLC system.

Keywords: Natural products, secondary metabolites, Alkaloids, Terpenes, biological targets.

INTRODUCTION

Natural products are also known as secondary metabolites; compounds that are naturally produced by biological organisms. Unlike primary metabolites, which are indispensable and absolutely required for survival (i.e. carbohydrates, fats, proteins, and nucleic acids), these natural products are not directly involved in the normal growth, development, or reproduction of the organism. While an absence of them does not result in immediate death, these secondary metabolites can affect the organism's fitness by playing a role in protection, competition, and inter- and intraspecies interactions (Croteau, Kutchan, and Lewis, 2000).

Natural products have a broad range of functions. These functions include agents that solubilize and transport nutrients, like siderophores which transport iron Fe^{+3} across membranes in bacteria and fungi; pheromones that act as social signaling molecules with other individuals of the same species, like queen mandibular pheromone secreted by the queen bee to feed her attendants that gives the colony a sense of belonging; communication molecules that attract and activate symbiotic organisms, like maltose released from chlorella algae to its cnidaria host and chemical agents that are used against competitors, prey, and predators, like the acacia trees which produce tannins in their leaves when they're preyed upon by giraffes. For many other compounds, the ecological and/or biological function is still unknown (Solomon, 2020).

Natural products are often structurally complex molecules that possess a well-defined spatial orientation. These compounds have evolved to interact efficiently with their biological targets, as their producers evolved alongside their target organisms. These natural products have effect in human health as well; in fact, it was the identification

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of cannabinoids in the 1960s that eventually led to the discovery and naming of the cannabinoid receptors in the 1990s. Research of these receptors and natural cannabinoids led to the development of drugs for stimulation of appetite and amelioration of nausea as well as symptomatic relief of cancer pain, proving that these natural products are a relevant starting point for drug development (Solomon, 2020).

Review Trends in Alkaloids and Terpenes

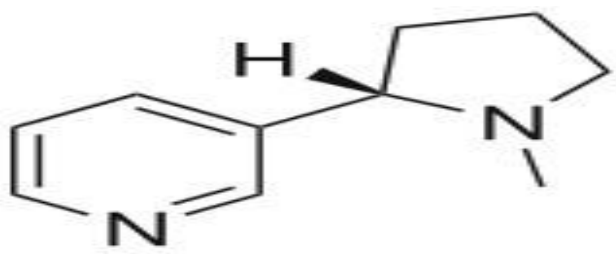
Alkaloids

Alkaloids are organic heterocycle constituents with nitrogen, plant secondary metabolite, most of them are toxic in nature; they contain at least one atom of heterocycle nitrogen. Alkaloids are the main secondary metabolites which have medical properties. Alkaloids can be used to avoid the several degenerative sicknesses by scavenging free radical or binding with catalyst, the oxidative reaction. Several studies indicated the alkaloids from several plants have a wide range of pharmaceutical application (Roy, (2017). Various compounds of alkaloids like coniine, atropine, nicotine, cocaine, codeine, morphine, quinine, strychnine, papaverine, and caffene. The effects of these constituents on human are healthiness (Lucy *et al.,* 2020). Alkaloids constituents basis on their biosynthetic precursor and heterocycle ring system, include indole, tropane, piperidine, purine, imidazole, pyrrolizidine, isoquinoline, pyrrolidine and quinolozidine alkaloids (Roy, 2017).

Alkaloids are broadly spread from vegetation, plants which are rich in alkaloids involve Papaveraceae family (Poppy), Solanaceae crops (tobacco, potatoes), and Rubiaceae (the quinine tree) (Isac *et al*., 1980). Numerous European scientists discover and isolated compounds including the isolation of xanthine (1817), strychnine (1818), atropine (1819), quinine (1820), and caffeine (1820) (Heinrich *et al*., 2012). Alkaloids include neuroactive molecules, like nicotine, and caffeine, and the antitumor vinblastine and vincristine. Alkaloids play as defense compounds in plants, well-organized against predators and pathogens due to their toxicity.

Diverse strategies are utilized to study alkaloid accumulation and metabolism. The effective method is gene expression monitor, concentration of precursors, and enzyme activities of the alkaloid itself through controlled occurrences the pathogens and herbivores or simulation of their occurrence by chemical, physical stimulation (Matsuura, & Fett-Neto, 2017).

Natural products of plants have been used by humans for thousand years, as drugs, foods, antioxidants, fragrances, flavors, insecticides, dyes, and pheromones, enhancing the health. Alkaloids are among the major groups of secondary metabolites. Their existence really diverse in terms of biosynthetic pathways and structure, involving more than 20,000 diverse molecules that are present in almost 20 % of recognized vascular plants (Yang & Stöckigt, 2010). Mechanism of action of the alkaloid is complex, meaning that, the toxicity observed in insects is not necessary similar to other animals. Key aspects associated to symptoms of toxicity involve the active metabolite amount, the organ which it is in contact, and specific features of the target organism. Understanding the metabolism and action of alkaloid may lead to beneficial molecules for human healthiness and harvest production. Important drugs of plant alkaloids involved morphine for treating severe pain; cephaeline and emetine like antidotes for intoxication; caffeine to stimulant effect; quinine used as antimalarial; antitumoral vinblastine, vincristine; camptothecin as anti-arrhythmic and ajmalicine as antimicrobials; sanguinarine and berberine antitussive (Yang & Stöckigt, 2010). Therefore, alkaloid compounds have a great importance in medicine; can be used as drugs against various sicknesses.



Structure of Alkaloid

Terpenes

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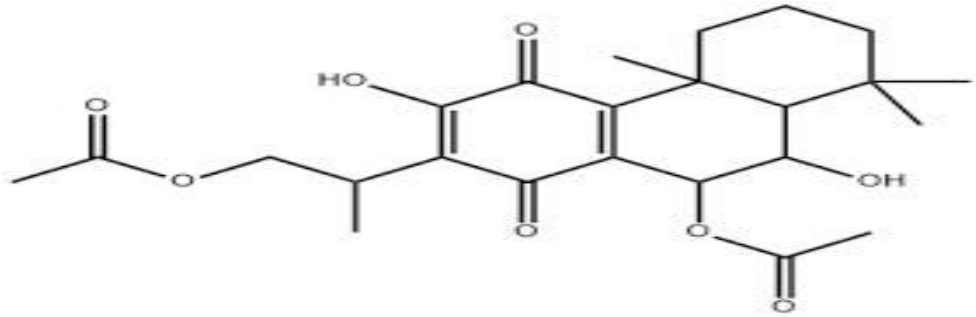
Terpene is the generic name of a group of natural products, structurally based on isoprene (isopentenyl) units. The term may also refer to oxygen derivatives of these compounds that are known as the terpenoids. The theory that provided the first conceptual framework for a common structural relationship among the terpenes was first formulated by Wallach in 1887 after carrying out structural investigations of several terpenes. His theory stated that terpenes can be viewed as being made up of one or more isoprene (2-methyl-1,3-diene) units joined together in a head to tail manner. Wallach's idea was further refined in the 1950 by Ruzicka's formulations of the biogenetic isoprene rule, emphasizing mechanistic considerations of terpene synthesis in terms of electrophilic elongations, cyclizations and rearrangements.

Terpenes or terpenoids are the major diverse group occurrence in plants naturally, depended on isoprene unit's number, can be classified in to mono, di, tri, tetra, and sesquiterpenes. They are mostly present in plants and make the chief constituent of essential oils from plants source, commonly plant terpenes sources are tea, cannabis, thyme, citrus fruits, and Spanish sage (Cox-Georgian*et al.*, 2019). Terpenes have several functions in plants like a signaling functions, pigments, solvents, flavoring, and have several medicinal applications (Yang *et al.*, 2012). Terpenes contains several remedial properties such as anticancer, antimicrobial, antiviral, antifungal, analgesic antihyperglycemic, antiparasitic, and anti-inflammatory, it is also utilized to improve skin penetration, avoid inflammatory sicknesses. In medicine, terpenes are used for several medical drugs (Franklin and Cunnington, 2001). Also against pathogens, herbivores, and organisms as mycorrhiza, and pollinators (Falara, 2014). Other researchers indicated the terpenes have a broad range of therapeutic uses as antiplasmodial, antimalarial drug. Monoterpenes exactly are broadly studied for their antiviral, antidiabetic, and anticancer properties in modern world. Many terpenes were broadly utilized in natural folk medicine. One of such terpene is curcumin which was evaluated as anti-inflammatory, anticancer, antioxidant, antiseptic, astringent, antiplasmodial, diuretic, digestive, and other properties (Cox-Georgian *et al.*, 2019). All plants produce several of hundred terpenoid compounds with characters that involve phytohormones, anti-oxidants, protein modification reagents, and more. Phylogenetically terpenoids are restricted and implicated the organism's attraction (Pichersky and Raguso, 2018).). Humans used several of terpenoid compounds to produce important compounds such as vitamin A from β-carotene.

Generally, terpenes in foods contain a main effect on our eating practice. Terpenoid as pigments, like bixin, astaxanthin, and lycopene, are utilized in the food manufacturing. Volatile compounds of terpenoid produce specific flavors to foods; ginger flavor is produced through zingiberene. Many herbs (as lemon grass), and spices (as saffron) have volatile terpenoids as chief flavor components, and alcoholic drinks (Stewart, 2013). Globally, sales pharmaceuticals drugs which depend on terpene were in 2002 nearly US $12 billion. Among these pharmaceuticals, antimalarial drug Artemisinin, and anticancer drug Taxol. Terpenoids show a broad range of biological application against cancer, inflammation, malaria, and a diversity of infectious illnesses (bacterial and viral). Natural product from the marine environment has hundreds of terpenoids compounds with different structures and has bioactivities, with more to be discovered in the future. (Cox-Georgian *et al.*, 2019).

Although total chemical synthesis plays chief role in the making of some terpenoid drugs, which contributed significantly to the progress of terpene-based drugs (Wang *et al.*, 2005). Terpenes or isoprenoids are organized in a regular head to tail. Squalene (unsaturated hydrocarbon present in humans, shark), the side chains of Vitamin A, E, K, are all compounds of terpenes. Terpenes produce fragrances which utilized as cosmetics, insect repellants, pollination, perfume preparation, and have several therapeutic values (Kandi *et al*, 2015). Most of these compounds are present at low levels in nature. Biological and metabolic engineering methods provide approaches to produce sufficient amounts of terpenoids for drug product (Proshkina *et al*, 2020).



Structure of Terpenes

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Isolation of Natural Products:

Unlike the medicinal chemist, who usually concentrates on a series of synthetic compounds of known chemical and physical properties and hence is able to master the limited number of separation techniques applicable to the specific chemotype, the natural product chemist must be prepared to deal with molecules of the whole spectrum of bioactive metabolites. These can vary in hydro- and lipophilicity, charge, solubility, and size. (McAlpine and Hochlowski, 1994). In general, the more hydrophilic metabolites may be candidates for ion exchange chromatography, reversed phase silica gel chromtography, or sizeexclusion chromatography on polysaccharide resins. The more lipophilic metabolites can be further purified by chromatography on normal phase silica gel, florisil, alumina, or lipophilicsize exclusion resins such as sephadex LH-20.

They may be also candidates for a variety of high-speed countercurrent techniques or chromatography on polyresins (McAlpine and Hochlowski, 1994). However, this branch of the main task of the natural product chemist was advanced to large extent. The production of many new packing materials, new isolation instruments with high degree of resolution and detection facilitated the chemist´s duty in this branch of the task. Some problems are still unresolved, for example, separations of individual pure compounds from mixture of cerebrosides, many trials were applied for achievement of this goal but unfortunately, all failed. The sphingolipids (cerebrosides) are easy to be separated as a mixture of closely similar chemical constituents from natural sources.

However, they are very difficult to be separated from each other or in another word they are very difficult to be separated and purified to analytical purity. The difficulty arises probably from their chromatographic properties being overshadowed by the polar nature of the glycoside, thus making these metabolites difficult to separate to analytical purity (Jenkins, 1999). Furthermore, the fact that, these metabolites are present oftenly as a series of very similar chemical nature, where the difference in most cases is one or two methylene groups, thus many of the published cerebrosides were reported as a groups of mixed compounds (e.g.,Jenkins 1999 & Inagaki, 2003). However, HPLC in reversed-phase mode is now the standard method for separation of molecular species of cerebroside mixture, often after benzoylation so that the lipids can be detected by sensitive UV spectroscopy. Mass spectrometric methods are now being used increasingly for charaterization purposes of such sphingolipids (Christie, 2003).

Characterisation of Natural Products:

This branch of the task is the first step in structure elucidation. Therefore it is very important for the natural product chemist; he has to spend his effort and time to drive the structure proposal in the right direction. In contrast, the medicinal chemist, who is dealing with the synthetic chemicals, has no need to spend his time and effort here because he knew previously what type of chemicals he is dealing with. In the fact, there are many simple and general chemical tests that are widely available and more applicable since a long time, for example, Molish's test for carbohydrates, Mayer`s and Dragendorf`s reagents for alkaloids, alkali solution (e.g. NaOH, and KOH) for both anthraquinones and phenolic compounds.

However, these reagents were intended to dissolve a small part of the problem. The phytochemist cannot decide precisely and also confirm the structure using these simple reagents. These reagents tell us about the general chemical class to which the isolated compound may be related. But in most cases they give no idea about the sub chemical class rather than the smallest details of the structure. Metabolite profiling is not an easy task to perform since natural products display a very important structural diversity. For each compound, the order of the atoms and stereo chemical orientations has to be elucidated *de novo* in a complex manner and the compounds cannot simply be sequenced as it is the case for genes or proteins.

Consequently, a single analytical technique does not exist, which is capable of profiling all secondary metabolites in the biological source (Wolfender, *et al*, 2005). However, the employment of advanced analytical and spectroscopic methods like UV and IR- spectroscopy solved such problems to a large extent, where they can give a good idea about the different substructures and/or functional groups of the structure. The hyphenated techniques coupled to HPLC give not only ideas about the details of functional groups, but also play an important role in what is called "Dereplication process" in order to avoid the tedious isolation of known compounds, and directed the chemist´s effort toward the targeted isolation of constituents presenting novel or unusual spectroscopic features (Wolfender *et al*, 2005).

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In this review of a certain study, a lot of chemical compounds that share the same chromophoric functions were examined by the hyphenated technique HPLC-UV-photodiode array detection (LC/UVDAD). This examination showed that, all chemicals having the same chromophoric functions will show the same UV spectrum with the same absorption maxima even though they are different in their additional non-chromophoric functions and their molecular weights.

Kahalalide F, (compound 1, m/z 1478), R (8, m/z 1520) and S (9, m/z1536), all having the same chromophoric function and therefore showing the same UV spectrum and having approximately the same absorption maximum at ~ 203 nm.

- Kahalalide E (2, m/z 836), Kahalalide D (2, m/z 596), N,N-dimethyl tryptophanmethylester. (6, m/z 246), indole-3-acetic acid (39, m/z 175), all having the same chromophoric function (tryptophan amino acid) and therefore showing the same UV spectrum and having approximately the same absorption maxima at ~ 220, 279 sh and 285sh nm.

- A group of 3-acyl indole derivatives, e.g., hyrtiosine A (13, m/z 191), 5-hydroxy-1Hindole-3-carbaldehyde (14, m/z 161),), indole-3-carbaldehyde (15, m/z 145) and 5-deoxyhyrtiosine A (16, m/z 175) all having the same chromophoric function (3-acylindole) and therefore showing the same UV spectrum and having approximately the same absorption maxima at ~ 210, 250, 275 and 300 nm. The same UV spectrum was obtained for the new indole derivative, isohyrtiosine A (17, m/z 191) with a little deviation in the UV-absorption maxima. Isohyrtiosine A showed an absorption maximum at 214, 241 and286 nm due to the presence of carboxyl group instead of a ketonic group.

- New purine derivatives, nigricines 1-3 (34, m/z 307), (35, m/z 279), (36, m/z 265), all having the same chromophoric function (2-oxo-purine) and therefore showing the same UV spectrum and having approximately the same absorption maxima at ~ 210 and 290 nm. The same UV spectrum was obtained for both nigricine 4 (37, m/z 281), and adenosine (30, m/z 267), with a little deviations in the UV-absorption maxima. Ashourine4 showed an absorption maxima at 214 and 315 nm due to N(7)-methylation and loss of one double bond at position 8, while adenosine showed an absorption maxima at 207 and 257 nm because it has no 2-oxo- group.

- p-Hydroxyphenyl acetic acid, and its methyl-, ethyl- and butyl esters, [(26, m/z 152) (27, m/z 166) (28, m/z 180) and (29, m/z 208)] all having the same chromophoric function (4-hydroxyphenyl) and therefore showing the same UV spectrum and having approximately the same absorption maxima at ~ 202, 224 and 275 nm. However, the compounds that have no chromophoric functions cannot be characterised by LC-UV hyphenated technique. Also, NMR spectroscopy plays a significant role in characterisation of natural products:

The peptide nature of isolated kahalalides (compounds 1-5, 8& 9) was suggested by:

1) From ^{1}HNMR, the presence of amide NHs resonating in the downfield region between 7.0-9.0 ppm, α-protons resonating around 4.0 ppm and the CH_3 groups resonating in the higher field region around 1.0 ppm.
2) From $_{13}$C NMR, The presence of amide carbonyls resonating around 170 ppm, α- carbons (sp3 methines) resonating between 50-60 ppm and methyl carbons in the higher region.
3) From a COSY, each amino acid could be distinguished by a sequential correlations beginning from the amide NH, through α-, β-, γ- to the methyl protons which could be confirmed by the total correlation spectroscopy (TOCSY).

- The cerebroside nature of compounds 32 and 33 (petrocerebrosides 1 and 2) were established from the 1HNMR which showed a triplet-like signal at δ 0.82 (terminal methyl) and broad singlet at δ 1.2 (long chain $(CH_2)_n$ groups for both fatty acids and long chain bases), the presence of several doublets in the region between 4.2-5.3 ppm indicated the presence of many OHs (of the sugar parts), the presence of multiplet signals between 3.35 and 5.0 ppm indicated the presence of sugar CHs. In addition to one amide NH in the lower field region rather than the sp^2 methines in aromatic region between 5.0 –9.0 ppm regions. This suggestion could be supported by the presence of amide carbonyl signal in 13CNMR at approximately 170.0 ppm.

- The steroidal nature of the isolated steroidal compounds and also the scalarane type sesterterpenoids could be suggested from ^{1}HNMR by the presence of a characteristic methyl around 1.0 ppm and presence of overlapping

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methylenes and sp^3 methines in the higher field region between 1.0 and 2.0 ppm, which was supported by a characteristic $_{13}$CNMR spectrum.

- p-Disubstituted phenyl group could be distinguished from ^{1}HNMR by the presence of two doublets inter-correlated at approximately 6.8 and 7.2 ppm (especially for 4-hydroxy-1-substituted phenyl group), for example, tyrosine-containing natural products (kahalalideB, compound 4), and p-hydroxy phenylacetic acid and its derivatives (compounds 26, 27, 28 and 29).

- 3-Substituted-indole could be easily distinguished from ^{1}HNMR by the presence of ABCD spin system in the aromatic reagion (approximately between 7.00 and 8.2 ppm) for proton resonances of H-4, H-5, H-6 and H-7, in addition to a relatively sharp singlet or in rare cases doublet with small coupling constant of H-2 between 7.0 and 8.0 ppm.

Furthermore the sharp singlet in the down field region between 10.0 and 12.5 ppm indicating the indole NH. The present examples are compounds 2, 3, 6, 15, 16, 38, and 39. In contrast 5-hydroxy-3-substituted indole displays an ABM spin system(approximately between 6.80 and 8.0 ppm) for proton resonances of H-4, H-6 and H-7 instead of the above ABCD spin system (see ^{1}H NMR of compounds 13, 14 and 17).

Mass fragmentation pattern, could play a significant role in characterisation of natural products:

- Comparison of the MS fragmentation of the isolated compound with those of the known authentic samples may be enough not only for characterisation of substructures but also for full structure elucidation of the compounds.
- MS fragmentation pattern may be helpful in characterisation of some substructures which seem difficult to be distinguished by other means, rather than they are also helpful in establishing the relation between the closely related natural products, for example, the characteristic loss of 42 mass unit through retro Diels-Alder fragmentation from the new purines, nigricines 1-4, (compounds 34-37) indicated the presence of 2-oxo-purine derivatives and consequently the loss of NCO fragment, thus, the closed relation between them could be established.

- MS fragmentation mechanisms (e.g. tandem ESI/MS and MALDI-TOF-PSD) are described as the methods of choice in characterisation and confirmation of the amino acid sequence of the isolated peptides.

Structure Elucidation of Natural Products:

After the characterisation of the isolated natural product and determination of the sub chemical class to which the compound is related, the phytochemist has to demonstrate unambiguously the small details of the substructures and consequently the elucidation of the complete structure. Structure elucidation of the isolated new natural product is still the bottleneck of the objective achievement. Actually, the new technology in the field of NMR spectroscopy (including 1D- and 2D- NMR experiments) and mass spectrometry facilitated the chemist´s effort in this part of the whole task. Furthermore, the presence of commercial and non-commercial computer-assisted structure elucidation (CASE) programs even though they are not widely available and less applicable nowadays but may be the method of choice in the near future. It is important to say that the interaction between a spectroscopist and a CASE system will remain important in order to generate the correct structure rapidly.

Therefore CASE will complement the skills of the spectroscopist, not replace them. The use of CASE system is likely to increase in the near future, and this will enable the bottleneck often caused by structure elucidation to be removed from the natural product drug discovery process (Jaspers 1999).

Conclusion

The modern techniques as well as the advanced instruments that employed in the discovery programm of bioactive natural product facilitated the task of the natural product chemists and permitted them to detect, target, isolate and elucidate the structure of the pharmacologically active natural product in significantly short times compared with that done in the not too distant past. The targeted natural product may show novel or unusual spectroscopic features and/or promising biological activities. These methodologies are now applicable to achieve the task without the need of tedious isolation procedures of known compounds. The application of these new methodologies

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resulted in saving the time, efforts and economy during the processing of the bioactive natural product drug discovery programs.

The present review deals with the application of the modern techniques including NMR, MS, and hyphenated HPLC systems as very efficient and applicable tools in the achievement of the above mentioned aim.

References

1. Ashour, M. and El-Sharkiya, Ä.A. (2006).Structure Elucidation of Bioactive Marine Natural Products using Modern Methods of Spectroscopy. J. Nat. Prod., 69, 1547.
2. Cox-Georgian, D., Ramadoss, N., Dona, C., & Basu, C. (2019). Therapeutic and Medicinal Uses of Terpenes. In N. Joshee, S. A. Dhekney, & P. Parajuli (Eds.), *Medicinal Plants* (pp. 333–359). Springer International Publishing. https://doi.org/10.1007/978-3-030-31269-5_15.
3. Croteau, R., Kutchan, T., M., and Lewis, N., G. (2000). Natural products (secondary metabolites). Biochemistry & Molecular Biology of Plants, 1250-1318.
4. Dewick, P.M.(1997) In: Medicinal natural products, a biosynthetic approach, third avenue, New York, USA, 153-173.
5. Exarchou, V., Krucker, M., Beek, T.A., Vervoort, J., Gerothanassis, I. P., and Alert K. (2005) Magn. Reson. Chem. 43, 681-687.and references therein.
6. Falara, V., Alba, J. M., Kant, M. R., Schuurink, R. C., &Pichersky, E. (2014).Geranyllinalool Synthases in Solanaceae and Other Angiosperms Constitute an Ancient Branch of Diterpene Synthases Involved in the Synthesis of Defensive Compounds. *Plant Physiology*, *166*(1), 428–441. https://doi.org/10.1104/pp.114.243246.
7. Franklin, L. U.; G. D. Cunnington, and D.Y. (2001).Terpene based pesticide treatments for killing terrestrial arthropods including, amongst others, lice, lice eggs, mites and ants.
8. Gerwick, W.H. and Bernart M.W. (1992) Pharmaceutical and bioactive natural products In:Marine biotechnology 1. (Attaway D.H. and Zaborsky O.R. eds) Plenum Press, NewYork.
9. Giersiefen, H., Hilgenfeld, R., Hillish, A.(2003): Natural products for lead identification: natureis a valuable resource for providing tools, In: modern methods of drug discovery (ed,Hillish A., Hilgenfeld R).
10. Gullo, V.P. (1994): In The Discovery of natural products with therapeutic potential (Gullo, V. P. ed.), Butterworth-Heinemann press.
11. Hanson, J.R., (1979). In Comprehensive Organic Chemistry, Ed. Barton, D.H.R et al., Pergamon, Oxford, Vol. 5, p. 989.
12. Heinrich, M.; J. Barnes; S. Gibbons, and E.M. Williamson. (2012). *Fundamentals of Pharmacognosy and phytotherapy, (2nd ed).* Churchill Livingstone, Edinburgh.
13. Isac, O.; K. Thiemer, and L. Szelenyi.(1980). Medicinal plants.
14. Kandi, S., Godishala, V., Rao, P., &Ramana, K. V. (2015). Biomedical significance of terpenes: an insight. *Biomedicine*, *3*(1), 8-10.
15. Lucy, B. J.-A., Angela, M. D., Miriam, G. T., &Nazizi, K. (2020). Anti-nociceptive effects of the hydroethanolic extract of Alysicarpusovalifolius in rodents. *Journal of Medicinal Plants Research*, *14*(5), 195–201. https://doi.org/10.5897/JMPR2019.6769
16. Matsuura, H. N., &Fett-Neto, A. G. (2017). Plant Alkaloids: Main Features, Toxicity, and Mechanisms of Action. In C. R. Carlini& R. Ligabue-Braun (Eds.), *Plant Toxins* (pp. 243–261). Springer Netherlands. https://doi.org/10.1007/978-94-007-6464-4_2.
17. Newman, D.J., Cragg, G.M., Snader, K.M. (2000). The influence of natural products upon drug discovery. Nat. Prod. Rep., 17, 215-234.
18. Pichersky, E., & Raguso, R. A. (2018). Why do plants produce so many terpenoid compounds? *New Phytologist*, *220*(3), 692–702. https://doi.org/10.1111/nph.14178.
19. Proshkina, E., Plyusnin, S., Babak, T., Lashmanova, E., Maganova, F., Koval, L., Platonova, E., Shaposhnikov, M., & Moskalev, A. (2020). Terpenoids as Potential Geroprotectors.*Antioxidants*, *9*(6), 529. https://doi.org/10.3390/antiox9060529.
20. Roberts, J.S. (1972). In Chemistry of Terpenes and Terpenoids, Ed. Newmann A.A. Academic Press, London, p. 88.
21. Roy, A. (2017). A review on the alkaloids an important therapeutic compound from plants. *IJPB*, *3*(2), 1-9.
22. Stewart A. (2013). *The drunken botanist: the plants that create the World's great drinks*. Chapel Hill, NC, USA: Algonquin Books of Chapel Hill.

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23. Wang, G.; W. Tang, and R. R. Bidigare. (2005). *TerpenoidsAs Therapeutic Drugs and Pharmaceutical Agents. In: L. Zhang; A.L. Demain (eds)* Natural Products. Humana Press.
24. Yang, J., Xian, M., Su, S., Zhao, G., Nie, Q., Jiang, X., Zheng, Y., & Liu, W. (2012). Enhancing Production of Bio-Isoprene Using Hybrid MVA Pathway and Isoprene Synthase in E. coli.*PLoS ONE*, *7*(4), e33509. https://doi.org/10.1371/journal.pone.0033509.
25. Yang, L., & Stöckigt, J. (2010). Trends for diverse production strategies of plant medicinal alkaloids. *Natural Product Reports*, *27* (10), 1469. https://doi.org/10.1039/c005378c

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