Research Paper

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Bioactive Natural Compounds from *Cactus Euphorbia Caducifolia*

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Abstract

In recent years, bioactive compounds are in high demand in the pharmaceuticals and naturopathy, due to their health benefits to human and plants. The bioactive of bioactive compounds. Bioactive compounds like alkaloids, steroids, compounds are extra-nutritional constituents present in small quantities in lipid rich foods and plant products. Endophytes are organisms that live inside healthy tissue of plant. Majority of endophytic bacteria showed beneficial effects like enhancement of biological nitrogen fixation, production of phytohormons, solubilization of phosphate etc. Endophytic bacteria, fungi and actinomycetes plays significant role in production flavonoids, quinols, phenols. Cacti have attracted special interest due to their particular traits, such as a spinosebody that stores water and photosynthesize with CAM metabolism. Such traits allow them to live in extreme environments and tolerate water stress.

Introduction

Endophytesan overview

Endophytes are group of microorganisms having the ability to enter inside the plant hosts colonizing the intercellular spaces and also the xylem vessels. Almost all plants species have been found to harbour endophytic bacteria or fungi (Sturz and Nowark 2000). Moreover, the colonization of endophytes in algae (Smith and Barko 1990; Stanley, 1992), mosses and ferns (Petrini et al. 1992; Premila et al., 1996) has been recorded. They prevail in a range of tissues types within a broad range of plants, colonizing the plant systemically with bacterial/fungal colonies.Endophytes can offer several benefits to the host plant, particularly growth promotion and protection from pathogens; and that under diverse environmental conditions bacterial endophytes are able to communicate and interact with the plant more efficiently than rhizospheric bacteria (Ali et al., 2012, Coutinho et al., 2015).They are ubiquitous, colonize most of the plants, and have been isolated from almost all plants examined till date. Endophytes are to live symbiotically within the plants. While growing inside the plant the endophytes shows no visible symptoms of infection and disease (Bacon and White, 2000). The term endophyte was first carried out by De Barry in 1879. There is general consensus that microbial communities make significant contributions to plant health. Large numbers of substances are derived from the plants and therefore serve as an important source for hunting biologically active endophytes. Endophytes can also be useful for the crop production. Biological strategieshave to be developed to increase crop productivity and to reduce the usage of chemical fertilizers and also environmental stress tolerance of plant. And these can be accomplished by the judicious application of strains of plant growth promoting endophytes (PGPE)(Saraf et al.,2018). Clay &Schardl (2002) recognized three types of clavicipitaceousendophytes, ranging from symptomatic and pathogenic species (Type I) to mixedinteraction and asymptomatic endophytes (Types II and III, respectively). Endophytes are hyperdiverse at the species level, phylogenetically rich, ecologically important, evolutionarily dynamic, and represent an under explored trove of taxonomic, genetic, and functional diversity. Endophytes have been found in every plant and lichen species, including wild, crop and forage lants in all teristrial biomes samples so far. Endophytes play a major role in physiological activities of host plants influencing enhancement of stresstolerance, nematode and disease resistance (Carroll,1988; Hallmann and Siora 1996; Azevedo et al 2000; Strurz and Nowark 2000).

Endophytes are rich in all terrestrial communities, boreal forests, an imperilled ecosystem of immense global importance and one in which their ecological associations, genetic diversity, and functional roles are largely unknown. Entophytes colonizing inside plant tissue usually get nutrition and protection from the host plant. In return they confer profoundly enhanced fitness to the host plant by producing certain functional metabolites. Endophyte containing plants grow faster than the non-containing ones (Cheplick et al., 1989). This effect is atleast in part due to production of phytohormones such as indole-3-acetic acid (IAA). Endophytes would have enhanced the hosts' uptake of nutritional elements such as nitrogen (Ries et al., 2000) and phosphorus (Gasoni and Gurfinkel, 1997; Malinowski and Belesky, 1999). Endophytes are viewed as outstanding source of bioactive natural products as many of them occupy literally millions of unique biological niches, growing in diversified environmental conditions. Thus, it would appear that these bio typical factors can be important in plant selection since they may govern the novelty and biological activity of the products associated with endophytic microbes. The symptomless nature of endophyte occupation in plant tissues has prompted focus on symbiotic relationships between endophytes and their hosts. Both fungi and bacteria are the most common microbes existing as endophytes. Selective microbial forms like mycoplasma and archaebacteria have not yet been reportedto exists in plants as endophytes (Strobel and Daisy, 2003).Endophytes are viewed as outstanding source of bioactive natural products as many of them occupy literally millions of unique biological niches, growing in diversified environmental condition. Endophytes promote the growth of plant in various ways, that include secretion of plant growth regulators via phosphate solublizing activity (Walelin et al., 2004), production of siderophores (Costa and Loper, 1994), supplying biologically fixed nitrogen (James et al., 1994) and enhancing hyphal growth and mycorhizal colonization (Will and Sylvia, 1990).In addition, endophytes are also reported to supply essential vitamins to plants (Rodelas et al., 1993) ,confer protection against plant pathogenic microorganisms via production of antibiotics (Struz et al., 1999) or synthesis of secondary metabolites (Long et al., 2005).

Plant under examination:*Euphorbia Caducifolia*

Euphorbia caducifolia is a plant of *Euphorbiaceae* which includes 300 genera and 7,500 species. It is a latex yielding plant, grows abundantly in arid and semiarid regions of western and central India. The phylloclades of this species are tough, succulent and fleshy, covered with divaricated sharp spiny stipules. *E. caducifolia* (ECL) is used by the local inhabitants for treatment of bleeding

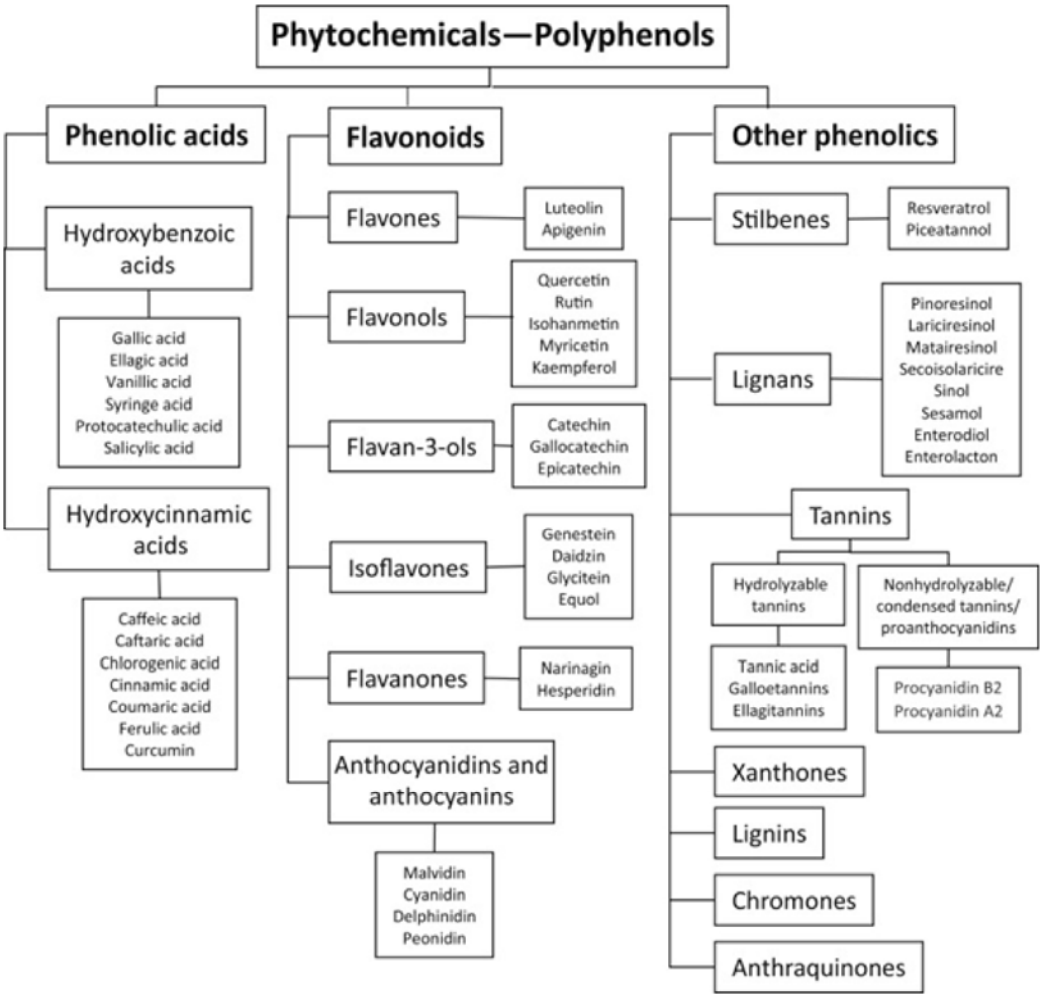
wound, cutaneous eruption and other skin diseases. Looking to the medicinal importance of the plant, it was considered worthwhile to scientifically evaluate the for the wound healing activity. *E. caducifolia* is observed to be used in 31 different disease conditions. It is exclusively used in skin ailments, cough, asthma and body pain. It is effective against gastro-intestinal diseases, gynecological and obstetrical problems, respiratory diseases, in musculo-skeletal disorders, ENT disorders, immune disorders and as an antidote in snake-bite, scorpion bite. *E. caducifolia* has also been reported for its wound healing, (Goyal, Nagori and Sasmal, 2012) antimicrobial, antibacterial, antifungal (Goyal, Sasmal and Nagori, 2012) and anti nematologiacal activities (Maqbool, Hashmi and Ghaffar, (1987). Dry environments have plants with special adaptations which enable them to live on nutrient-poor soils, in high temperatures, and with a scarcity of water. One of the most important features is the ability to establish symbiosis with different microorganisms. Desert plants have several types of adaptations that help them conserve water. Like a leathery or waxy coating on the leaves and stems reduces evaporation. Thick stems or other plant parts provide water storage space. Small leaves or spines (modified leaves) reduce the surface area of the plant exposed to the sun. Spines and fine hairs reflect heat and reduce the air flow over the plant's surface.

Phytochemicals in cactus

Endophytes are recognized as the potential sources of novel natural products for exploitation in medicine, agriculture and industry with more bioactive natural products isolated from the microorganisms (Bacon and White, 2000; Strobel and Daisy, 2003; Kumar and Sagar, 2007).Cactus species are important natural and economic resources in desert environments due to their adaptability and rich phytochemistry.These plants are rich in vitamin C, carotene and secondary metabolites such as polyphenols.(Stinzing and Carle,2005)these polyphenols includes mainly phenolic acids, flavonoids, tannins, saponins and alkaloids.These compounds are helpful to plants as well as human.Research have shown that plant based foods high in flavonoids can reduce mortality rates by 25% as well as significantly decrease the instances of myocardial infarction. They are also known to improve cardiac function, decrease anginas and cholesterol level. Phenols reduces the toxicity of streptozotocin by neuytalizing the free radicals produces in the pancreatic cells. Phytochemicals generally originated from the plant source are nothing but the bioactive

compounds also known as secondary metabolites. There are two types of metabolites produced in plants viz. Primary metabolites and Secondary metabolites. Primary metabolites are important for the plants regular metabolism such as growth and development. Secondary metabolites produced by plants may have little need for them. These are synthesizing in almost all parts of the plants like bark, leaves, stem, root, flower, fruits, seeds etc. During past several years, phytochemicals have been used worldwide as the traditional herbal medicine. Because of this pharmaceuticals industry as well as researchers put a greater emphasis on the phytochemical studies. Also, these phytochemicals present in different plant parts are used up by the local people for healing of certain disorders. These are also widelyused in the field of agriculture. Secondary metabolites are economically important in the production of drugs, flavor, and fragrances, dye, and pigment, pesticides and food additives. Many of the drugs that are derived from the secondary metabolites are simple synthetic modifications or copies of the naturally obtained substances. Plants are the tremendous source for the discovery of new products with medicinal importance in drug development. Secondary metabolism, particularly in the possibility of altering the production of bioactive plant metabolites by means of tissue culture technology. Plant cell and tissue culture technologies can be established routinely under sterile conditions from explants, such as plant leaves, stems, roots and meristems for both the ways for multiplication and extraction of secondary metabolite in plant cell suspension cultures has been reported from various medicinal plants, and bioreactors are the key step for their commercial production. Based on this lime light, the present review is aimed to cover phytotherapeutic application and recent advancement for the production of some important plant pharmaceuticals. Secondary metabolites, though not essential for growth of an organism, play an adaptive role in functioning as the defense compound or the signaling molecule during ecological interactions and environmental stresses. Endophytic microorganisms produce low-molecular weight secondary metabolites that include antimicrobial compounds, phytohormones, or their precursors, vitamins like B_{12} (Ivanova et al. 2006) and B1 (Mercado and Bakker 2007), bioprotectants (Trotsenko and Khmelenina 2002). Several secondary metabolites are alkaloids, steroids, terpenoids, polyketones, flavonoids, quinols and phenols. These compounds also have important role in therapeutic applications such as anti-cancer, antioxidant, antimicrobial, anti-inflammatory, and immunosuppressive agents (Korkina 2007). Endophytes produce alkaloids

as the secondary metabolites that have diverse biological potential as anti-fungal, anti-cancer, and anti-viral agents (Silva et al. 2007).

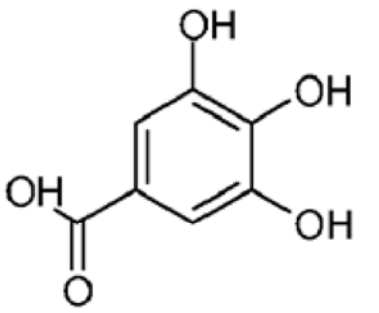


Phytochemicals helps plant to survive under harsh conditions, protects against herbivores and other insects. Alkaloids, phenols, saponins, tannins, terpenoids, carotenoids, flavonoids, steroids etc are known as phytochemicals.

Tannins

The term tannin was first introduced by Seguin to indicate various plants extracts which have the capacity to convert hides and skins into leather (Seguin A,1796). Vegetable tannins are important

as retanning agent in the leather production and have been recognized as an important tanning agent in non-chrome tanning. Tannins are water-soluble phenol derivatives naturally synthesized and accumulated by higher plants as secondary metabolic products. From a chemical point of view, tannins are polyphenols with molecular weights between 500 and 3000 Da. In complexes with saccharides, alkaloids, and proteins its molecular weight can increase even up to 20,000 Da (Haslam Edwin, 1998) that exhibit characteristic reactions for phenols. The chemical structure of tannic acid depends on the plant species producing the compound. Currently, more than 8000 different tannins have been isolated and chemically characterized. However, there are certainly many more tannins with chemical structures that have not been precisely determined yet. All tannins have some common features, which enable classification of these types of compounds in two main groups, three types of hydrolysable tannins: gallotannines, ellagitannines, and complex tannins(sugars derivatives—mainly glucose, gallic acid, and ellagic derivatives) and condensed tannins(nonhydrolysable) called procyanidins containing condensed carbon chain typical for flavonoids (Khanbabaee and Van Ree, 2001). Condensed tannins are much more resistant to microbial degradation than hydrolysable tannins and exhibit stronger antibacterial, antiviral, and antifungal activity.



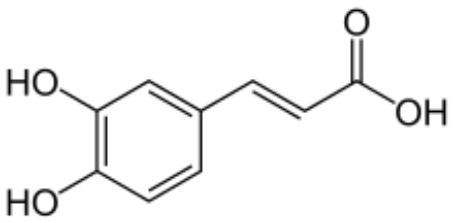
Gallic acid

Phenols

Plant phenolics" and "polyphenols" are secondary natural metabolites arising biogenetically from either the shikimate/phenylpropanoid pathway, which directly provides phenylpropanoids, or the "polyketide" acetate/malonate pathway, which can produce simple phenols, or both, thus producing

monomeric and polymeric phenols and polyphenols, which fulfill a very broad range of physiological roles in plants. Higher plants synthesize several thousand known different phenolic compounds. The ability to synthesize phenolic compounds has been selected throughout the course of evolution in different plant lineages, thus permitting plants to cope with the constantly changing environmental challenges over evolutionary time. Plant phenolics are considered to have a key role as defense compounds when environmental stresses, such as high light, low temperatures, pathogen infection, herbivores, and nutrient deficiency, can lead to an increased production of free radicals and other oxidative species in plants. Both biotic and abiotic stresses stimulate carbon fluxes from the primary to the secondary metabolic pathways, thus inducing a shift of the available resources in favor of the synthesis of secondary products. Phenolic phytochemicals are known to exhibit several health beneficial activities such as antioxidant, anti-inflammatory, antihepatotoxic, antitumor and antimicrobial (Hertog, 1995; Rice-Evans et al., 1996; Middleton et al., 2000).

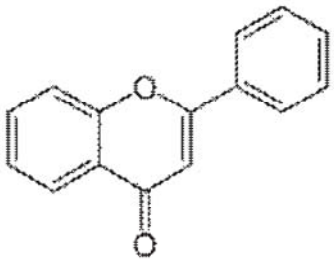
Caffeic acid



Flavonoids

Flavonoids are plant pigments that are synthesized from phenylalanine and generally display astonishing colours in the ûowering parts of plants. Flavonoids comprise a large group of polyphenolic compounds that are characterized by a benzo-y-pyrone structure, which is ubiquitous in vegetables and fruits. Besides the irrelevance in plants, ûavonoids are important for human health because of their high pharmacological activities as radical scavengers. It's potential health benefits arising from antioxidant activities has been increased the interest of extraction of these phenolic compounds. In general, all ûavonoids are derivatives of the 2 phenyl chromone parent compound composed of three phenolic rings referred to as A, B, and C rings all of which exhibit various levels of hydroxylation and methylation. Flavonoids are the most common and widely

distributed group of plant phenolic compounds, occurring virtually in all plant parts, particularly the photosynthesising plant cells. They are an integral part of both human and animal diets. Being plant phytochemicals, ûavonoids cannot be synthesized by humans and animals. Flavonoids found in animals are considered to originate from the plants that animals feed rather than being biosynthesized in situ. More than 5000 different plant-derived ûavonoids have been isolated from various plants. They are classiûed into at least 10 chemical groups. Flavanones, ûavones, isoûavonoids, ûavans (ûavanols), anthocyanins, and ûavonols are particularly common in the diet. Quercetin, kaempferol, and myricetin are the three most common ûavonols. Flavanones are mainly found in citrus fruits and ûavones in celery. Some ûavonoids have been reported to possess a variety of biological activities, including antiallergic, antiinûammatory, antiviral, antiproliferative, and anticarcinogenic activities, in addition of having effects on mammalian metabolism.

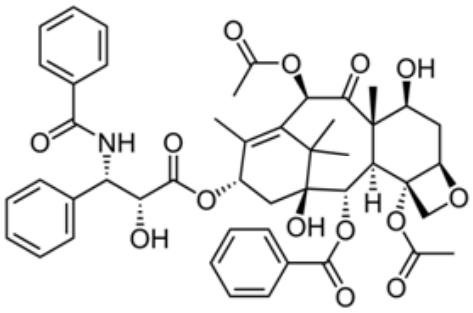


Flavone

Terpenoids

Terpenoidsarefeasibly the most structurally varied class of plant natural products. The name terpenoid or terpene derives from the fact that the first members of the class were isolated from turpentine. All terpenoids are derived by repetitive fusion of branched five-carbon units based on isopentane skeleton. These monomers generally are referred to as isoprene units because thermal decomposition of many terpenoid substances yields the alkene gas isoprene as a product. The smallest terpenes contain a single isoprene unit; as a group, they are named hemiterpenes (half-terpenes). The best-known hemiterpene is isoprene itself, a volatile product released from photosynthetically active tissues. Plant terpenoids are used for their aromatic qualities and play a role in traditional herbal remedies. Terpenoids contribute to the scent of eucalyptus, the flavors of cinnamon, cloves, and

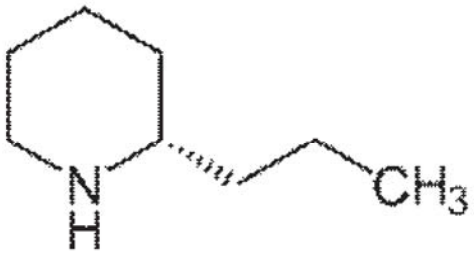
ginger, the yellow color in sunflowers, and the red color in tomatoes.[3] Well-known terpenoids include citral, menthol, camphor, salvinorin A in the plant *Salvia divinorum*, the cannabinoids found in cannabis, ginkgolide and bilobalide found in *Ginkgo biloba*, and the curcuminoids found in turmeric and mustard seed. Camphor and camphene are significant teroenoids. They are both naturallyoccurringterpenoidsprimarilyfoundun laurel trees (*Cinnamonumcamphora*). Camphor is a pungent terpenoid used primarily as a fragrance additive and in cooking recipes, while camphene, a monoterpene,is often used as a camphor substitute. It is sold as a color-less crystal with a strong, camphor-like scent, and is an ingredient in many foods, ointments, and topical creams. In Asia, amphor has long been used in the treatment of pain, swelling, and inflammation and served as a topical analgesic, providing a cooling effect for sore muscle tissue (Scott, R. Camphor and Camphene.)



Taxol

Alkaloids

Alkaloidsare a class of naturally occurring organic nitrogen-containing bases. Alkaloids have diverse and important physiological effects on humans and other animals. Well-known alkaloids include morphine, strychnine, quinine, ephedrine, and nicotine.Alkaloids are found primarily in plants and are especially common in certain families of flowering plants. More than 3,000 different types of alkaloids have been identified in a total of more than 4,000 plant species.Ancient people used medicinal plant extracts such as opium poppy (Papaver somniferum), Sarpagandha root (Rauwolfia serpentina) as purgatives, antitussives, sedatives, and treatments for a wide range of ailments, including snakebite, fever, and insanity.

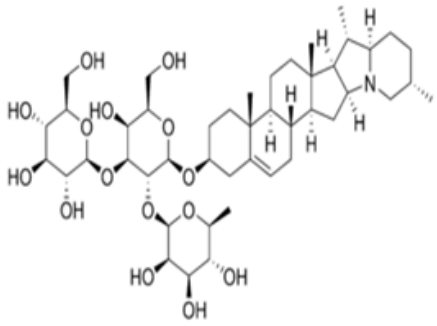


Coniine

Alkaloid-containing plants were mankind's original "materia medica." Many are still in use today as prescription drugs. One of the best-known prescription alkaloids is the antitussive and analgesic codeine from the opium poppy. Plant alkaloids have also served as models for modern synthetic drugs, such as the tropane alkaloid atropine for tropicamide used to dilate the pupil during eye examinations and the indole-derived antimalarial alkaloid quinine for chloroquine.

Saponins

Saponins are naturally occurring surface-active glycosides produced by plants, lower marine animals, and some bacteria. Chemically, they generally occur as glycosides of steroids or polycyclic triterpenes. Because of their lyobipolar properties, they are able to interact with cell membranes and are also able to decrease the surface tension of an aqueous solution. This activity is the reason for the name "saponin", derived from the Latin word "sapo", which refers to the formation of a stable soap-like foam in aqueous solution. Saponins occur constitutively in a great many plant species, in both wild plants and cultivated crops. In cultivated crops, the triterpenoid saponins are generally predominant, while steroid saponins are common in wild plants used as herbs. Plant saponins have been shown to inhibit cholesterol absorption from the intestinal lumen in experimental animals and consequently to reduce the concentration of plasma cholesterol. The triterpenesaponins called ginsenosides are the major active constituents in ginseng.Saponins have been associated with numerous pharmacological actions, the important ones being permeabilizing of cell membrane, lowering of serum cholesterol levels, stimulation of luteinizing hormone release leading to abortifacient properties, immunomodulatory potential via cytokine interplay, cytostatic and cytotoxic effects on malignant tumor cells etc.



Solanine

Identification methods of phytochemicals

Chromatography techniques

Chromatography is a technique where the molecules are separated based on their size, shape and charge. During chromatography analyte in solvent and move through solid phase that acts as a filtering material. As molecule proceeds further through molecular sieve it gets separated. Paper and thin layer chromatography are the chromatographic techniques which readily provides qualitative information and through which it become possible to obtain quantitative data.

Which includes paper chromatography, TLC, HPLC, GC etc.

Paper chromatography

In paper chromatography a sheet of paper is used for the inert phase. One of the advantages of paper chromatography is that separations are carried out simply on sheets of filter paper, which acts as both support as well as medium for separation. Another advantage is the considerable reproducibility of Rf (retention factor) values determine on paper. In paper chromatography, filter paper used as solid phase, which is inert phase. A sample is placed near the bottom of the filter paper. Then this filter paper is placed in chromatographic chamber with solvent. The solvent moves forwards by capillary action carrying soluble molecules along with it. Low porosity paper will produce a slow rate of movement of the solvent and thick papers have increased sample capacity.

Thin layer chromatography (TLC)

The first practical application of thin layer chromatography was given by Stahl. Compared to

paper chromatography, the special advantage of TLC is the versatility, speedy and sensitive. TLC is an adsorption chromatography where samples are separated based on the interaction between a thin layer of adsorbent attached on the plate. The technique mostly employed for the separation of low molecular weight compounds.

Gas chromatography (GC)

Gas chromatography is a method for the separation of volatile compounds. In this method, species distribute between gas and a liquid phase. The gas phase is flowing and the liquid phase is stationary. The rate of migration for the chemical species is determined through its distribution in the gas phase. For example, a species that distributes itself 100% into gas phase will migrate at the same rate as the flowing gas, whereas, a species that distributes itself 100% into stationary phase will not migrate at all. Species distribute themselves partly in both phases will migrate at an intermediate rate. Gas chromatography involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is then transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.

High performance liquid chromatography (HPLC)

HPLC is an analytical technique for the separation and determination of organic and inorganic solutes in any samples especially biological, pharmaceutical, food, environmental, industrial etc. Another name for HPLC is high – pressure liquid chromatography, separates compounds on the basis of their interactions with solid particles of tightly packed column and the solvent of the mobile phase. Modern HPLC uses a non-polar solid phase, like C18 and a polar liquid phase, generally a mixture of water and another solvent. High pressure up to 400 bars is required to elute the analyte through column before they pass through a diode array detector (DAD). A DAD measures the absorption spectra of the analytes to aid in their identification. HPLC is useful for compounds that cannot be vaporized or that decompose under high temperature, and it provides a good complement to gas chromatography for detection of compounds.

Methods for detection

Fourier-transform infrared spectroscopy (FTIR)

Fourier- transform infrared spectroscopy is a valuable tool for the identification of functional groups present in the plant extract. It helps for identification and structure determination of the molecule. Samples for FTIR can be prepared in a number of ways. For liquid samples, the easiest is to place one drop of sample between two plates of sodium chloride. The drop forms a thin film between the plates. Solid samples can be milled with potassium bromide (KBr) to and then compressed into a thin pellet which can be analysed. Otherwise, solid samples can be dissolved in a solvent such as methylene chloride, and the few drop of solution is then placed onto a single High Attenuated Total Reflectance (HATR) plates and spectra was recorded in terms of percentage transmittance. The peaks at specific wave number were assigned by bonding and functional group as per the reference given in Varian FTIR instrument manual.

Nuclear Magnetic Resonance Spectroscopy (NMR)

Nuclear Magnetic Resonance Spectroscopy gives physical, chemical and biological properties of matter. One dimensional technique is routinely used but the complicated structure of the molecules could be achieved through two-dimensional NMR techniques. Solid state NMR spectroscopy is used for the determination of molecular structure of solids. Radiolabelled C NMR is used to identify the types of carbon are present in the compound. H-NMR is used to find out types of hydrogen are present in the compound and to find out how the hydrogen atoms are connected.

Mass spectrometry (MS)

Mass spectrometry is a powerful analytical technique for the identification of unknown compounds, quantification of known compounds and to elucidate the structure and chemical properties of molecules. Through MS spectrum the molecular weight of sample can be determined. This method mostly employed for the structural elucidation of organic compounds, for peptide or oligonucleotide sequencing and for monitoring the existence of previously characterizes compounds in complex mixtures with a high specificity by defining both the molecular weight and a diagnostic fragment of the molecule simultaneously.

Cactus as Antioxidant

Cactus has been used in traditional folk medicine because of its role in treating number of diseases and condition including diabetes, hypertension, hypercholesterolemic, rheumatic pain, gastric mucosa and asthma, Cactus fruits contain bioactive compounds. It has been found that fruit juice contains a rich variety of natural antioxidants. An antioxidant is a molecule that inhibits the oxidation of other molecules. The free radicals produced during oxidative processes release highly reactive compounds generated in the body as byproducts of normal processes. Insufficient levels of antioxidants or inhibition of the antioxidant enzymes cause oxidative stress that may damage or kill DNA cells. Plant or animal foods contain a variety of nutrient/non-nutrient antioxidants, such as glutathione, vitamin C, Vitamin A and vitamin E. They are investigated for the prevention of disease such as cancer, coronary heart disease and even altitude sickness.

Conclusion

This research review's purpose to help the reader understand different aspects of phytochemicals isolated from endophytes of cactus *Euphorbiacaducifolia.* And it is concluded that the desert plant is having Phenols, Tannins, Saponins, and Alkaloids as bioactive natural compounds. These compounds help plant to survive under harsh condition and also protects against herbivores. They are also having good dietary supplements which reduce hypertension, acts as anticancer, antiulcerogenic and antioxidant agents. Cactus is a desert plant which grows under harsh arid environment. Results from this study suggest that they are important producers of many novel compounds that are significant in the field of medicine, pharma, agriculture and industries.

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