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ISOLATION AND STRUCTURAL CHARACTERIZATION OF NOVEL TERPENOIDS FROM DIFFERENT PLANT SPECIES: A REVIEW.

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Abstract:

Terpenoids are the most essential compounds biosynthesized by plants as secondary metabolites. It is a diverse and vast class of naturally occurring organic compounds and are the derivatives of terpenes having simple and multi-cyclic rings in their structure. These are though found in small/trace amounts in plants, but they play an indispensable role in the survival of plants in the environment. These are used by the plants as a shield against internal and external stresses and for many of the basic functions in the development and growth and by humans as anti-bacterial agents, as anti-carcinogenic agents, as flavors, fragrances and drugs. This review paper will explain the extraction and isolation of terpenoids from different parts of plants i.e. leaves, stem, roots and other vegetal parts of plants. Further the review will focus on the structural characterization of terpenoids extracted from plants by different researchers and the methods they used for the separation and structural characterization of these compounds and their structures drawn with the help of NMR data. The extracted and characterized compounds were terpenoids, triterpenoids, nortriterpenoids, monoterpenes, sesquiterpenes and macro cyclic monoterpenes. These compounds were checked for their medicinal activity, cytotoxicity and their bioassay were also considered, where these compounds showed that they can be used as medicinal compounds as well as the bioassay confirmed their role as protective agents of the plants. Overall, eleven research articles have been considered in this review and the focus is on the novel terpenoids that have been extracted, isolated and characterized by different means. Apart from novel terpenoids the other known compounds have been studied and their activity has also been reported.

Keywords: *Terpenoids, Characterization, Extraction, NMR, Plant.*

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INTRODUCTION:

The survival of plants in ecosystem is based upon the metabolites that are synthesized by plants. These metabolites are of two types: i.e. primary metabolites and secondary metabolites. The primary metabolites are vital for the growth and survival of plants whereas secondary metabolites have specific chemical and biological features. Secondary metabolites are necessary for plant survival in the ecosystem in a sense that they suppress and hinder the growth of other vegetation which may be harmful for the major plant. These metabolites thus are involved in interaction of the plant with environment and these metabolites also have the property to protect the plant from many stresses. Due to the above properties these compounds are often referred to as allelochemicals (Pagare, 2015).

The allelochemicals or secondary metabolites are though not essential for plants to live but are very important in a way that they help plants to interract with environment and are used as a shield (Calera, 1995; Abrosca, et al., 2005; Macias et al, 2002).

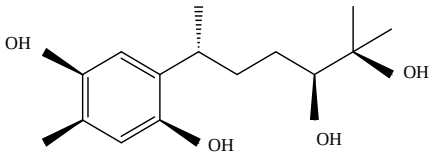
Secondary metabolites, when produced in plants have unique carbon skeleton and properties. They have low molecular weight and are organ tissue and cell centric and protects the plants against both biotic and abiotic stresses of all kinds. The abiotic stresses includes attacks from bacteria, fungi, moisture, nematodes, insects, grazing, etc. (Bhansal, 2015) These metabolites are used as drugs, flavours, fragrances, dyes and insecticides by humans due to their greater economic value. On the basis of their structure and biosynthesis, these compounds can be classified as polyketides, phenypropanoids and terpenoids. (Yazaki,2016). Out of the above, terpenoids are the largest specialized metabolites of plants classified on the basis of five carbon units in their skeleton. Terpenoids in large amounts with diverse structures are produced by plants as secondary metabolites that act as defensive agents and have the character to repel a large number of feeding insects and even mammals. They are classified on the basis of presence of five carbon units which are hemi (C_5), mono (C_{10}), sesqui (C_{15}), di (C_{20}), sester (C_{25}), tri (C_{30}) ad tetra (C_{40}) terpenoids. (Yazaki, 2017). They have wide spectrum of biological activities including potential allelopathy. Pyrethroid which are also known as monoterpene esters are present in Chrysenthemum species is a natural insecticide and used on commercial scale due to the reason that it has less toxicity to mammals and is less persistent in environment. Further limonene, myrecene and a pinene are found in conifers and pines and leguminous trees posses diterpene (abietic acid) that act as a defensive agent of this class of plants as well as reduces the risk of feeding and grazing. (Ncube, 2008;Krolicka, 2006;Pagare 2015). Enzyme Terpene Synthase (TPS) makes up their basic structure and are further modified by chemical process such as hydroxylation, dehydrogenation etc (Dudareva, 1996).

Similarly, many of the terpenoids have commercial value because they posses flavour and fragrances (e.g. menthol, sclareol, etc.) and are used in foods and cosmetics and the others have the quality of flavour of fruits such as linalool. Few of the secondary metabolites have medicinal value as well and are used as anti malarial, anti- carcinogenic, diuretic and anti bacterial (Aharoni, 2005). However, these metabolites are present in traces and low amount in plants. Due to their presence in low concentrations, their structure, extraction and characterization have always remained a subject of intrest and many researchers have worked on their structure and chracterization (Brusotti, 2013). These compounds have been extracted from different plants and their leaves by one or the other pathway available. Therefore, this review paper will focus on the different methods used by reasearchers in characterizing terpenoids and their isolation in order to get the knowledge of the structures of terpenoids and their existence in the floral parts as well as to analyze the methods and select the feasible and easiest method of their separation and characterization.

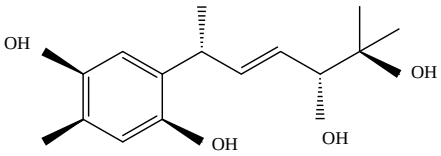
LITERATURE REVIEW:

Macias et al. (2004) with his team has carried out the work on the extraction of terpenoids from the dried leaves of sun flower (*Helianthus annus L.)* and found that the sun flower is reservoir of terpenoids specially sesquiterpenoids which are biologically active and allelopathic. The analysis was done and along with already reported species, four novel compounds of terpene group were also reported. These were two sesquiterpenes, a binorsesquiterpene and 7,11-hellianane. The separation of the compounds was carried out from the dried leaves of sun flower by using flash chromatography and column chromatography. Overall, after the separation, 13 terpene compounds were separated in fractions from the extract including leptocarpin, helliannuols A, C, D, E, F, G, H, I and L, sesquiterpene lactones annuolide E and sesquiterpene helibisabonol A & B. The one- and two-dimensional NMR spectral process was used to identify, elucidate and characterize the compounds. Different signals were obtained for different compounds and that were checked with

reference ranges and analyzed. The annuinone E compound separated as colorless oil and the structure was further elucidated by using infrared spectroscopy and it revealed that the structure of the compound contains an OH and COH group in the skeleton. After complete characterization of all the products, the bioassay was performed using the seeds of *Triticum aestivum L.* and it was reported that zero value represented control, the negative value corresponded as inhibitor and the positive value of the bio assay was reported as stimulus. So, as a result, the sequiterpenes including lactones laptocarpin, helliannuol A and helibisonol A among the separated terpenes were found to have negative bioassay value and thus are inhibitors to *T. aestivum L.* seeds. Figure 1 shows the chemical structures of hellibisonol A and B.



hellibisonol A



hellibisonol B

Figure 1: Structures of two novel terpenoids from the extract of sunflower leaves.

Calis et al. (2001) and his team from Turkish and Zurich University worked together on the extraction and characterization of glucosides a derivative of terpenes from the fruit of *Capparis spinosa* which is a native species of Turkey. The natives used the plant as anti-constipation agent, diuretic and a tonic and due to these properties, the plant was a valuable one. So, the team worked on the plant material to check the terpene derivatives and other organic compounds. The mature fruit of the plant for the preparation of extract was stored at –20 °C and was then crushed and homogenized and the mixture was passed through a column of SiO_3 (column chromatography) and the portions were obtained thus from the extract. Further, in order to clarify and elucidate the structures of the extracted compounds, the fractions were treated by using NMR spectroscopy techniques including HMBC, HSQC and ^{13}C NMR. The data from NMR revealed three (6S)-hydroxyl-3-oxo-α-ionol glucosides and a prenyl glucoside. For the complete understanding of their structures, the chiroptic and chemical evidences were also used. Along with the above terpene group, compound corchoionoside C (6S, 9S)-roseoside was also separated and its structure was thus elucidated. The ionol glucoside derivative was separated as colorless amorphous solid and showed UV spectrum value of 234 and infrared absorption at 1660 cm^{-1}, which corresponded to the presence of ketone group and the molecular formula of the compound characterized, was $C_{19}H_{30}O_9$. The other compounds isolated were not terpenes and thus are not described here.

Jakupovic et al. (1988) carried out the work on the isolation and characterization of glaucolides and guaianolidesfrom an African native plant species *Artemisia afra.* This specie has the characteristic that it contains special oils. The plant was grown specially and later dried for the preparation of extract. Thin layer chromatography, high performance liquid chromatography and flash chromatography techniques were employed on the extract to purify and separate the mixture into fractions and purify these obtained portions. During the separation, eighteen of the earlier known gluatanolides were obtained from the aerial parts. Futher, five glaucolides (Figure 2) were reported from this specie which are the derivatives of terpenes. The guatanolides were derivatives of one another and were isomers based on the position of hydroxyl groups whereas for glaucolides the structure (I) in Figure 2 after the analysis on NMR gave the idea of presence of a diacetate of a germacranolide and thus on the basis of NMR data the compound was named as lactone artemesia glaucolide. Further, the NMR spectrum for the structure (II a & b) were elucidated at high temperature, but still due to spin decoupling their structures could not be separated as there were isomeric double bonds as well as acetoxy groups at different carbons. Similarly, the ^1H NMR of compound (III) gave signals of the exomethylene protons and olefinic methyl. Structure (IV & V) in Figure 2, indicated the presence of eudesmanolide and α-cyperone respectively along with 12–hydroxyl groups. So, the separation of oxygenated guainolides was the character of this specie, but the isolation of glaucolide was unusual. The reported glaucolides has no oxygenated function in plants and after analysis were considered to be important in chemotaxonomy because during analysis many transformations were reported as well.

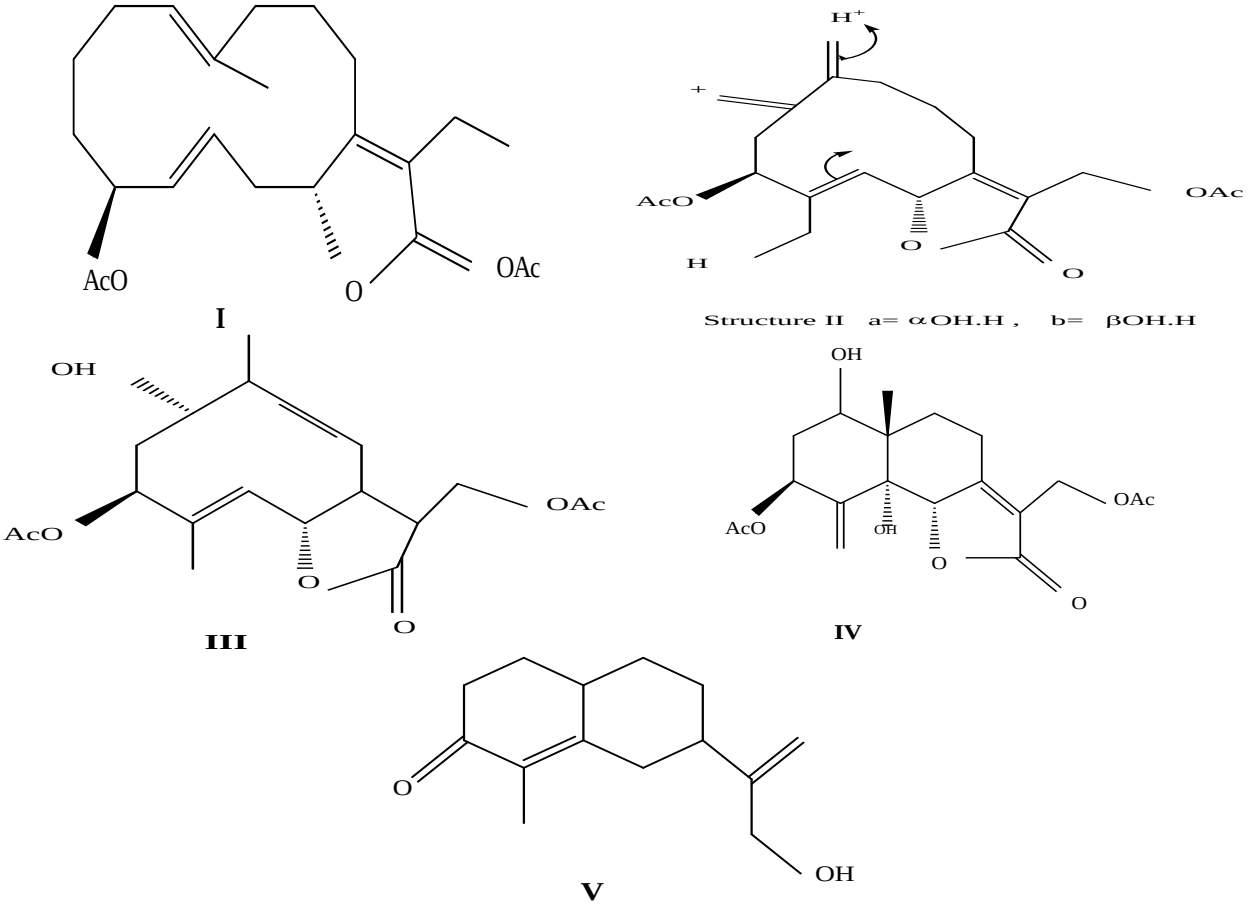


Figure 2: Structure of the five glaucolides from *Artemesia afra*

Abebayehu et al. (2016) has isolated and evaluated the structure of terpenoids from an Ethiopian croton specie commonly called as Bissana *(Croton macrostachyus)*. This plant was reported to have been used by the local populace for the treatment of many diseases like stomachache, headache, anti-diabetic, anti-malarial and even as a hypertensive agent. The phytochemical study of the plant leaves has shown presence of terpenes along with other thirteen chemicals. The fractions were separated by chromatographic techniques (thin layer and column) and the signals for terpenoids were analyzed on nuclear magnetic resonance spectroscopy ^{13}C NMR and DEPT–135. The data revealed that tri terpenoids containing mono terpenoids and sesquiterpenoids compounds from croton macrostachyus. After careful analysis and characterization, the pentacyclic tri terpenoid in Croton specie was reported as novel compound and one other tri terpene was successfully identified, but the test for phytotoxicity was not performed and the molecular formulas were not clearly mentioned in the paper. It was further reported that these compounds can further be analyzed and characterized by using sophisticated NMR techniques. The overall impact of the terpenoids present in Bissana on health was also not clarified.

D'Abrosca et al. (2005) has isolated and characterized different terpenoids from the leaves of Green Cestrum *(Cestrum Parqui)*. This plant is an ornamental shrub introduced from South America and is now naturalized and widely distributed. It is very toxic to animals and feasible environment for the plant in dense masses. Earlier, twelve C_{13} iso-prenoids have been separated from the leaves by spectroscopy. When analyzed on *Lactuca Sativa L.*, these compounds showed toxic effects on the growth and germination of that plant. Further, the leaves of the plant were powdered at –80 °C and extracted with alcoholic solution in a separatory funnel and the sample then was dried with sodium sulfate and concentrated in vacuum yielding the sample for experiment, this sample was then used for the isolation, phytotoxicity and structural characterization of terpenoids. Nine polyhydro

oxylated terpenes in which five were C_{13} nor-isoprenoids, 2 sesquiterpene, one spirostane and a pseudosapogenin were reported. Using NMR methods (TOCSY, HSQC, HMBC, NOESY, ROESY and COSY), the structures of these compounds were elucidated and as well as the terpene portions were analyzed phytochemically.

During the analysis they identified four novel structures of terpenes along with the others. The first among the novel compounds has the molecular formula of $C_{16}H_{30}O_4$ (Fig. 2) on the basis of the spectral lines and the analysis of the compound. Two Olefins protons were present in the structure. The data corresponded with the compound (3S, 5R, 6R, 7E, 9R)-3,5,6,9–terahydroxy-7–megastigmene. The second novel compound was identified and characterized having a sesquiterpene structure with the molecular formula $C_{15}H_{22}O_2$ (Fig, 3) which indicates the presence of non-saturation in molecule. The NMR analysis showed that the compound has four methylenes, four methines and four tetrasubstituted carbons. The third structure with molecular formula $C_{27}H_{44}O_5$ was identified with NMR data signals as 5α–spirostane-3β,12β,15α–triol. This compound was novel and reported for the first time although a terpene, spirostanol glycoside was already isolated from the same plant earlier (Baqui, 2001). Lastly, the fourth compound which was identified by MALDI-MS experiments having a molecular formula $C_{38}H_{62}O_9$ which shows pseudomolecular ion. This pseudo compound with ^{13}C NMR data was psedosapogenin glucoside 26–O–(3–isopentanoyl)–β–D–glucopyranosyl-5α–furost–20 (22)–ene-3β, 26–diol. It was the tricyclic sesquiterpene that was separaeted from the leaves of Green Cestrum. They further analyzed and taken the bio assay of these compounds to check their phytotoxicity on lettuce plant at different molar concentration ranges and their activity was phytotoxic like a herbicide pendimethalin. Literature data has revealed that the effects of this phytotoxicity will be on the cell division and elongation which would be hindered to a good extent (Hess. F.D, 1997).

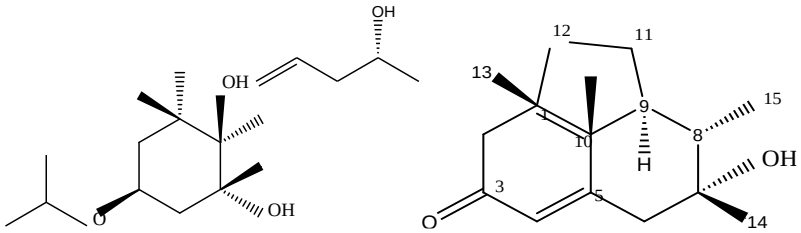


Figure 3: Structures of the two novel compounds separated from *Cestrum parqui*

In another research paper, the professors of American university have studied a weed (Mugwort) for the secondary metabolites and volatile allelopathic compounds. Mugwort *(Artemisisa vulgaris)* is one of the worst weed that affects nursery production. It was found to be home to many volatile allelochemicals. These chemicals were extracted from the tissues of fresh leaves and separated by gas chromatography. Later, mass spectrometry was used to characterize the compounds obtained from Mugwort. Many compounds were isolated and characterized including volatile terpenes. They were α-pinene, β-pinene, camphor, D–limonene, β– mycerene, eucalyptol, santolina triene and camphene. All these terpenes have less than 15 carbon atoms and are very small in size and thus are volatile. The *Artemesia vulgaris* plants from which these compounds were isolated were of three distinct types i.e. taller, shorter and highly branched by gas chromatography. During the study, it was revealed that terpene concentration in the leaves at earlier stage of growth was more as compared to the later stage. These terpenes were than subjected to mass spectrometry to characterize them. The foliage of Mugwort from which these were separated aged between 60 days to 120 days. Further, these compounds were analyzed for their phytotoxicity. It was reported that mycerene, β–pinene and limonene had no effect on test species. Euclaptol showed no phytotoxicity but caused shoot length elongation whereas, α–pinene was found to be a stimulator for cress redicle growth and camphor suppressed redicle and shoot growth. All the above monoterpenes were volatile and had great affinity for organic matter and thus were termed as lypophillic monoterpenes. The phytotoxicity varied from specie to specie and allelopathic nature depended upon the age of the foliage. So, the concentration of the terpenes was inversely proportional to the age of foliage and with higher age the phytotoxic effect also decreased. The presence of bioactive monoterpenes corresponded to their potential role in the plant growth and survival in a specific habitat. To end with, the weed was studied for volatile compounds and found to have eight different monoterpenes having very less phytotoxicity, but were important for the survival and growth of *Artemisia sp.* Their structures are illustrated as below in Figure 5 (Barney et al., 2005).

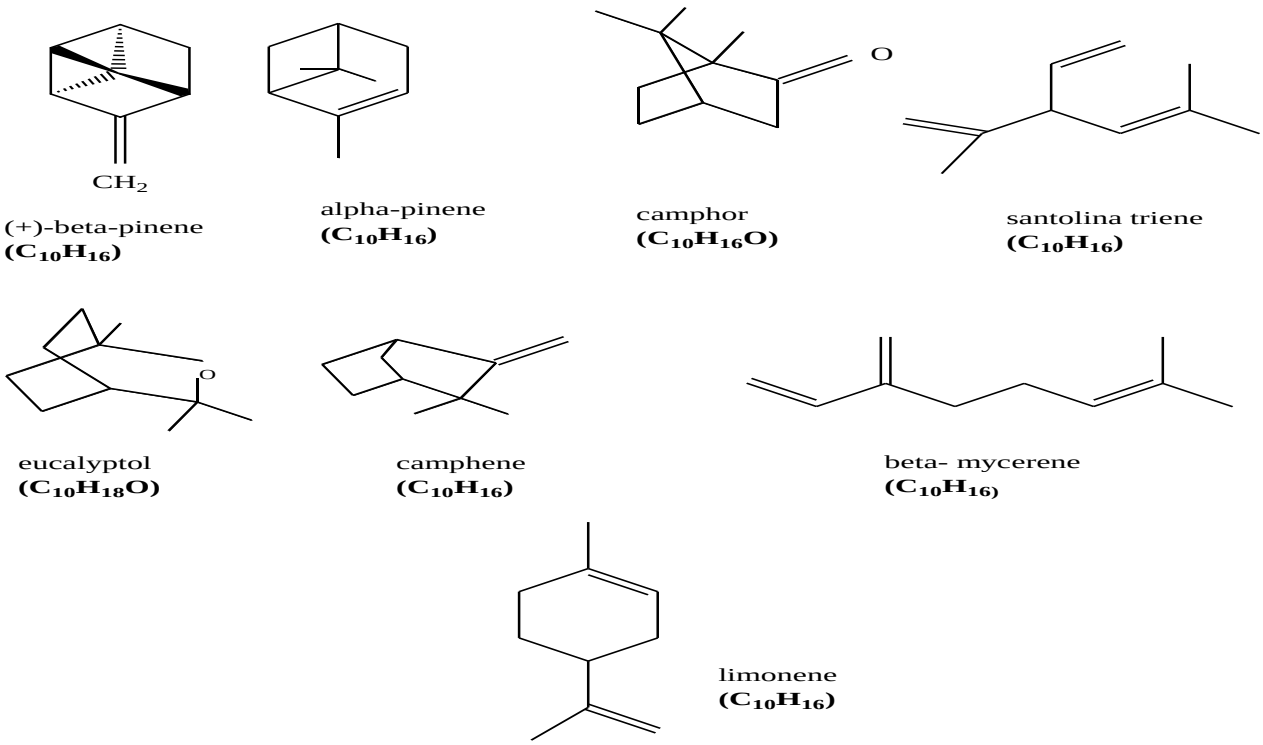


Figure 5: Structures of the eight monoterpenes isolated from *Artemisia vulgaris. (Mugwort)*

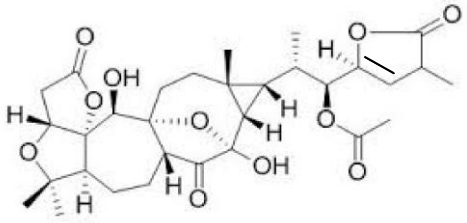
Huang et al. (2007) worked on *Schisandra* specie for the isolation of bioactive triterpenoids. *Schisandra chinesis* is a medicinal plant, which was used as sedative agent by natives of China, Japan and Korea. This is a climbing plant and the extraction and isolation of compounds was carried out by using the foliage and stem. The compounds were separated by different chromatographic techniques and were further characterized by using NMR-IR and mass spectrometry and their available methods of identification and characterization of unknown compounds.

During the study, almost 40 compounds were separated from the plant extract, which were found to be highly oxygenated with diverse and distinct carbon skeleton. All the compounds separated were characterized as nortriterpenoids. Due to the presence of these nortriterpenoids further study revealed that the plant contains dibenzocyclooctadienelignans, which had medicinal value and could be used as anti-hepatic, anti-cancer and as well as anti-HIV. As for as the isolated triterpenoids are concerned, most of them were of schisanartane carbon skeleton, which is new and diverse family of cycloartanes having octa cyclic backbone. The unprecedented ring structures and the feature of high oxygenation make these triterpenoids different from other naturally occurring terpenoids and from those extracted from other plants. The analysis resulted in the separation and characterization of henridilactone D, micradilactone and lancifodilactone C. Four other novel bioactive and oxygenated norterpenoids were also identified. The nomenclature of these four compounds was pre-schisanartanin and schindilactone A, B & C.

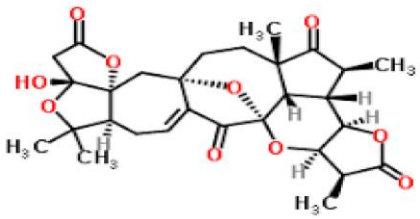
The pre-schisanartanin has an unusual structure and skeleton, which gave a new area of study in triterpenoids biosynthesis. The characterization of this compound was done using HPLC (HRESIMS) and the molecular formula for this colorless crystal was $C_{31}H_{40}O_{11.}$ The infrared spectra showed the presence of OH (hydroxyl) and COH (carbonyl) groups. The ^{13}C NMR data also confirmed a ketone in the structure of pre-schisanartanin.

Further, the three schindilactones i.e. A, B and C were analyzed and characterized using 2D NMR and X-ray experiments to confirm their structures. Among them schindilactone A and B showed similar molecular formula ($C_{29}H_{34}O_{10}$) but the spectral data showed that these may be epimers of carbon 20. Whereas, the fourth compound schindilactone C resembled to lancifodilactone C and henridilactone D and the data revealed the presence of a five membered α, β –unsaturated–α–methyl–γ–lactone ring. Due to presence of the above a OH and ether group is also a possibility in the structure. All the

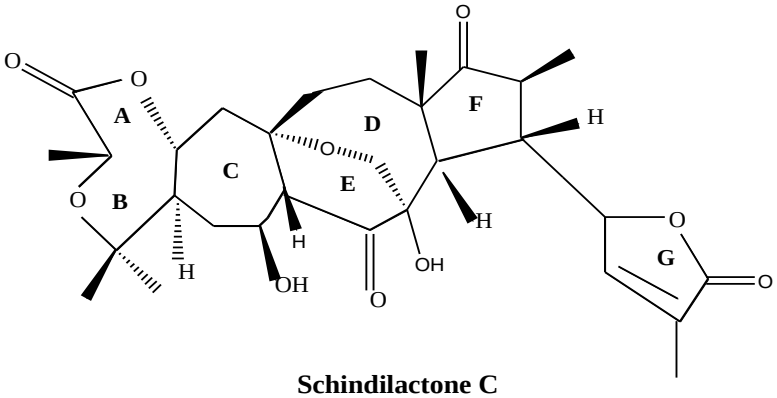
above compounds were specially tested and assayed for anti-carcinogenic character but showed no activity of inhibiting those cells. Additionally, the authors have encouraged that *Schisandra chinesis* must be further analyzed for more varieties of terpenoids as the plant had the potential to produce more terpenoid varieties. The structures of four novel compounds are shown in Figure 6.



Pre-schisanartanin



Schindilactone A & B
A = alpha CH_3
B = beta CH_3



Schindilactone C

Figure 6: Structure of four novel compounds from *Schisandra*.

Huang et al. (2013) in his second work has further studied *Schisandra chinesis* and isolated many miscellaneous terpenoids from it. In this paper, the plant species were collected from different geological zones. They have focused their study on terpenoids extraction with different, novel and unique structures. For this purpose, different species were studied of this genus. But *Schisandra chinesis* was extensively studied. The extract from the stem and foliage was used, analyzed and investigated for novel and known terpenoids. An earlier study on this plant species from a same region gave interesting results. Schisandra nortriterpenoids (Huang et al., 2007) had unique structural characterization explained earlier. Similarly, this study has further illustrated new terpenoid compounds with novel carbon skeletons. These skeletons included 3,4–seco–21,26–olide–artane, schisanartane, 18–norschiartane, 18-abeo–schiartane, pre-schiartanin, ursane, abietane,3,4–seco–lanostane, pre-schianartane, menthane and clovane. The structures after characterization showed that few of them have simple and small carbon skeleton like ursane, menthane etc. whereas, the other were found to have complex and diversified structures due to large number of carbon atoms and also involving different isomeric configurations. All the above nortriterpenoids were identified with the acetone extract of *schisandra chinesis*. The structures of few compound is shown in Figure 7.

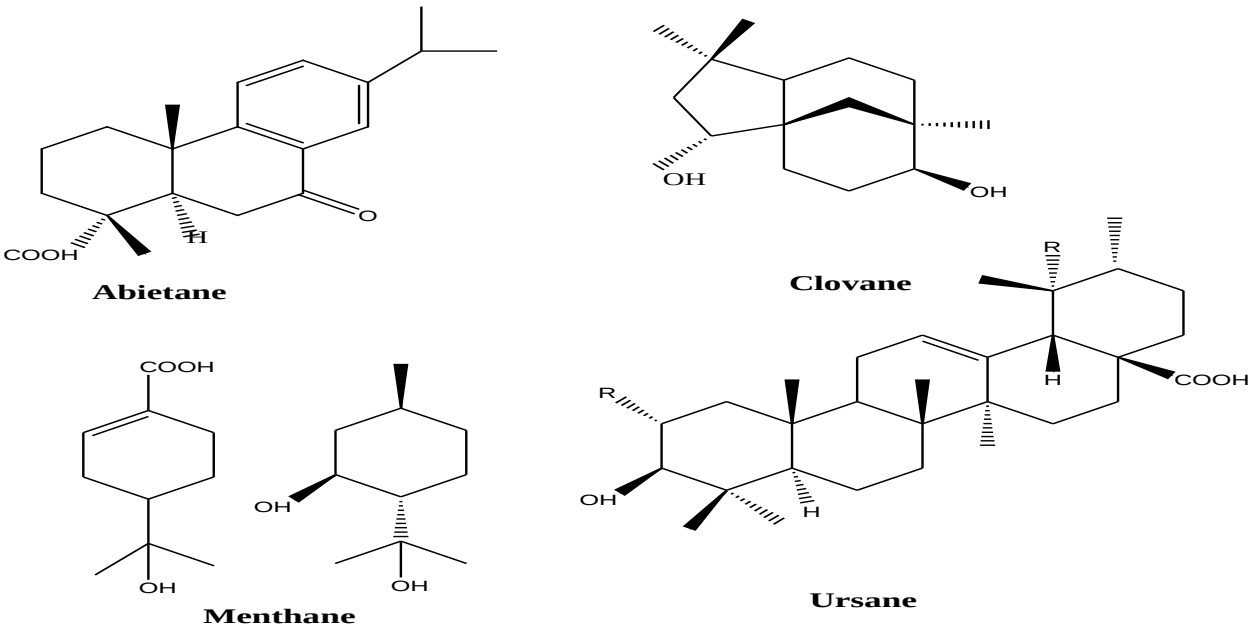


Figure 7: Structures of compounds from *Schisandra chinesis.*

In this research eight triterpenoids were novel, and these were separated by spectroscopy and also analyzed for their cytotoxicity and potent anti-HIV activity. These compounds were found to be very diverse in their skeleton and also had isomeric properties. The techniques of NMR used in the isolation were diverse for different compounds including ^{13}C NMR, ^{1}H NMR, DEPT NMR, COSY, HSQC, HMBC NMR, ROESY and 2D NMR data. Apart from NMR, mass spectrometric technique (HRESIMS) was also utilized and the data was thus used for structural characterization. Out of eight novel compounds two (wuweizidilactone G & H) were obtained as white solid with molecular formulas $C_{35}H_{44}O_{13}$ and $C_{28}H_{36}O_{10}$ respectively. Their characterized structures are shown in Figure 8.

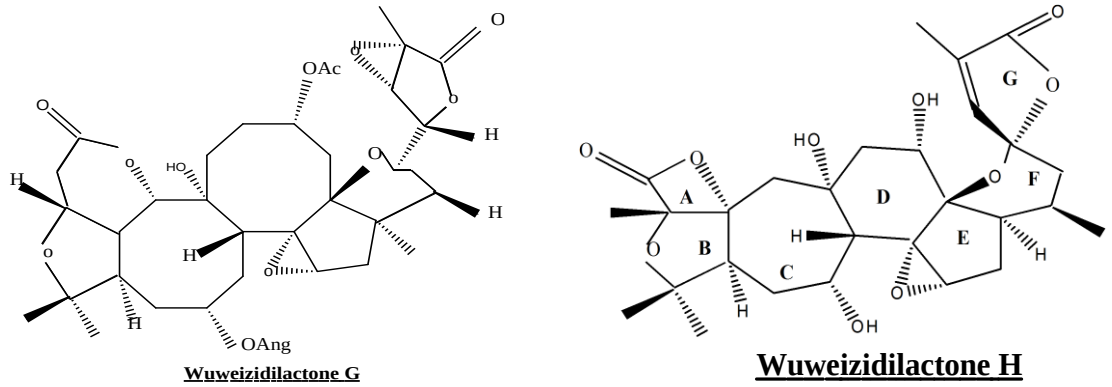


Figure 8: structure of the two wuweizidilactones.

Along with above two, another novel compound that was separated as white solid was characterized by using mass spectrometry and the molecular formula was evaluated to be $C_{29}H_{36}O_{11}$. Similarly, a compound with molecular formula $C_{29}H_{36}O_{11}$ was isolated as amorphous powder and two other novel compounds with molecular formulas $C_{29}H_{36}O_{10}$ and $C_{29}H_{34}O_{11}$ were also found and separated from the extract by using NMR. All the above four novel species were named as Schindilactone D, E, F & G respectively due to their similarity in their carbon numbers as well as closeness in their structures. Their structures are shown in Figure 9.

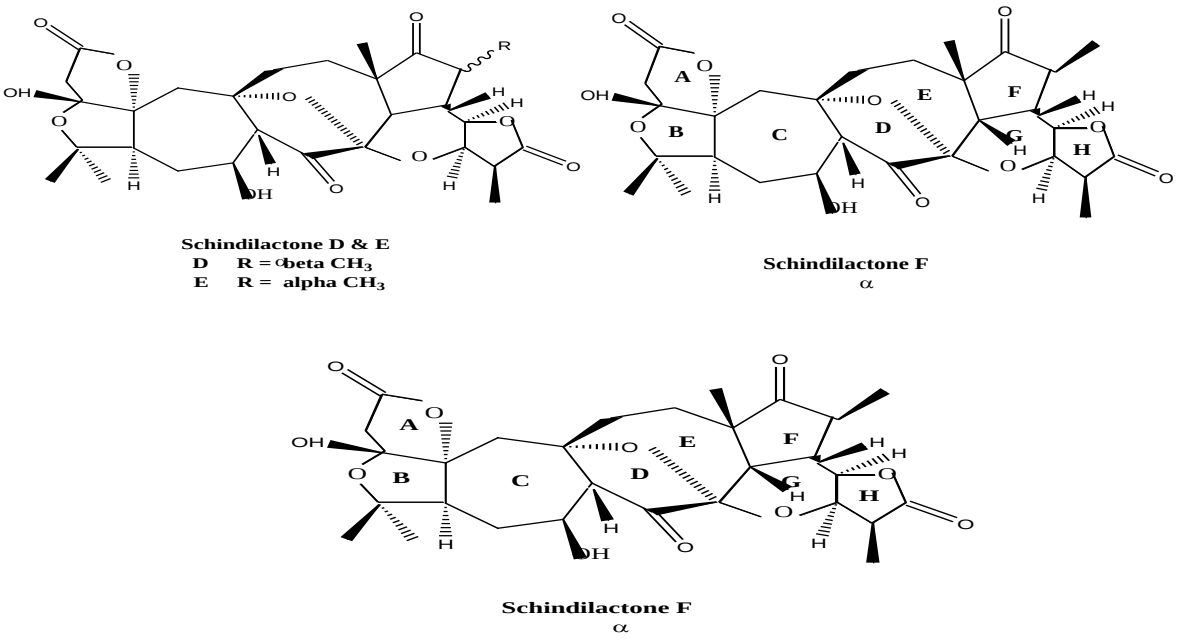


Figure 9: Structures of Schindilactone D, E, F & G.

Additionally, with the help of mass spectrometry (HRESIMS) and ^{13}C NMR another novel triterpenoid was characterized having molecular formula $C_{31}H_{42}O_{11}$ and it was given the name of pre-Schisanartanin B. The structure was a combination of different carbonyl, lactone carbons, a ketone and acetyl group. The last and eighth novel compound with 10degree unsaturation was identified by NMR-IR spectral data with molecular formula $C_{30}H_{42}O_5$, which corresponded to the derivative of 3,4–seco–cycloartane and was thus given the name wuweizilactone acid. Their structures are illustrated in Figure 10.

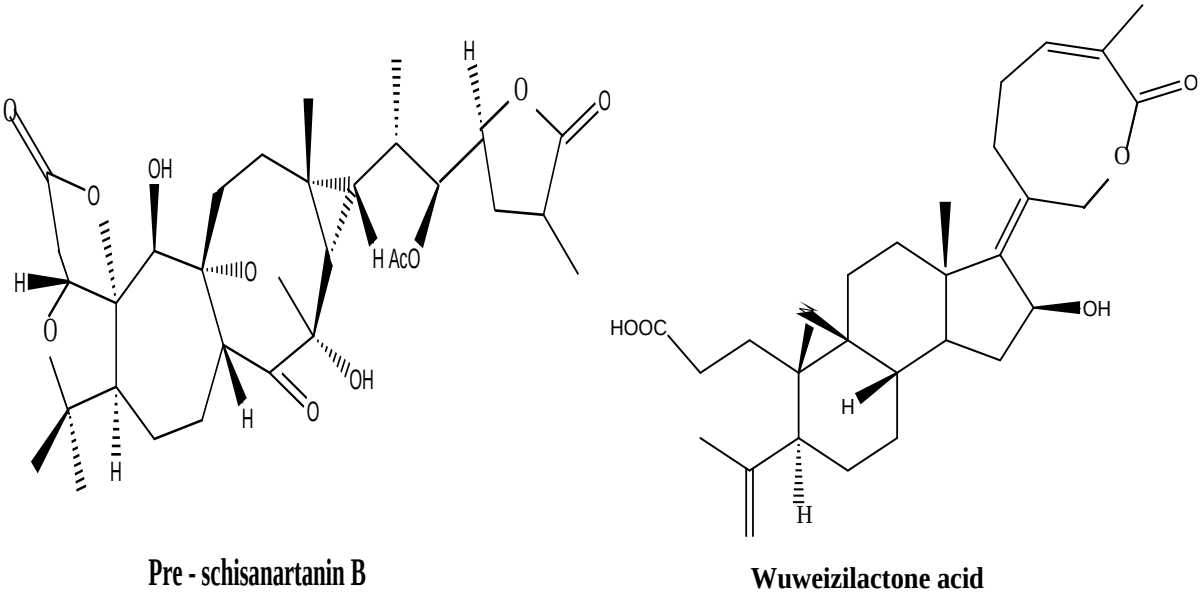


Figure 10: Structures of pre-schisanartanin B & Wuweizilactone acid.

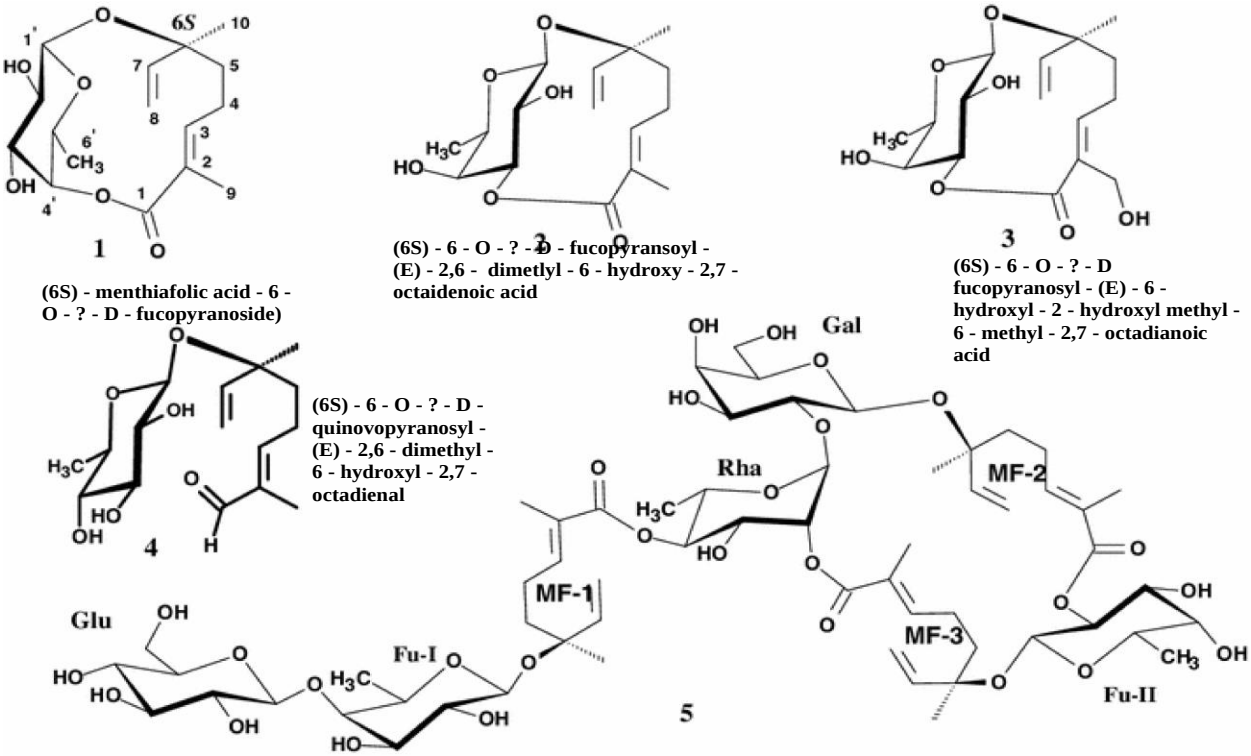
Overall, *Schisandra chinesis* of family Schisandraceae was found to be rich in bioactive terpenoids of diverse character and structure along with other bioactive compounds. The new

nortriterpenoids obtained from this study were thought to be beneficial and worth for further investigation.

Marzouk et al. (2012) has worked on the extraction, separation and characterization of macrocyclic monoterpenes from a Fabaceae specie (*Parkinsonia aculeata L.)*. The monoterpenes were glycosides which were found to be bioactive. *Parkinsonia aculeata L* is a thorny shrub commonly called as Jerusalem thorn and has the value of being a shady and medicinal plant. It is used in many parts of the world as anti-bacterial, anti-typhoid and anti-diabetic. For the isolation and extraction, the foliage (leaves) and small twigs were used as extract. In the study eleven known metabolites with phenolic properties were isolated which were three (3-ortho glycosylflavonols), Five (C–glycosylflavones), a diosmetin, an esculatin and a para–hydroxy bezoic acid. Their structures were evaluated by using spectroscopy (UV, MS, ^{1}D NMR & ^{2}D NMR). The study focused on five novel macrocyclic monoterpene glycosides along with other known compounds.

All the five novel compounds showed when characterized showed monoterpene behavior. The first compound was separated as white powder and was observed carefully using ^{1}H NMR, ^{13}C NMR, DEPT, COSY, GHMBC and GHMQC to structurally characterize the novel compound and was observed that the structure has monoterpenoidal aglycone, olefinic methylene, a sugar (β-6-doxygalactopyranoside),α-β-unsaturated COOH and carbonyl carbon. The structural data confirmed the novelty of the compound having macrocyclic features. Thus, the compound was named as Parkinsene A. [(6S)–menthiafolic acid–6–O–β–D–fucopyranoside)]

The other extract was off white powder and was analyzed as inseparable mixture of three monoterpene-O–glycosides. Chromatography (TLC & Column) as well as spectral data of these compounds showed the presence of many compounds including fucose and quinovose sugar, free carboylic groups, aldehyde, carbonyls etc. On the basis of spectral peaks and data analysis all the three in the mixture were found to be new compounds and were thus named as Parkinsene B, C & D. The last novel compound also showed the same chromatographic behavior of monoterpene glycoside but had a decreased R_f value with high polarity than other compounds. The structure when analyzed was a mixture of many secondary structures including sugars (rhamnose, galactose, glucose & fucose), menthiafolic acid, 6S–(E)-menthiafolic acid, hydroxymenthiafolic acid, methylenes, methines, methiafolic acid and deoxy sugars etc. Due to the presence of these signals, the structure was very diverse and this was named as Parkinsene E. (Molecular Formula $C_{60}H_{92}O_{28}$). Afterwards, the pharmacological study was also done for these extracted monoterpenes from *Parkisonia aculeate L.* and they showed activity as anti- pyretic, anti-inflammatory, anti- diabetic, hepato-protective and analgesics.



PARKINSENE E

Figure 11: Structures of novel terpenoids from *Parkinsonia aculeata L.*

Maldonado et al. (2013) had worked on isolation of sesquiterpenes lactones from the foliage and leaves of *Kaunia lasiopthalma Griesb.* This plant is the member of Astraceae family, which has only 14 members and they are found in Bolivia and other South American countries. This is basically a shrub having white purple flowers and called as Cochabamba by natives and they use it to treat headaches and inflammations. From this plant species, Gutierrez and his team isolated nineteen guaiane sesquiterpene lactones. In this paper they searched for biologically active chemical constituents (sesquiterpene lactones). The isolated and characterized compounds were eighteen known and two novel compounds (eudesmanolides). The known compounds included already isolated eudesmanes, flavones, guaiane and germacrane sesquiterpenes. These compounds were extracted from the leaves and flowers of *Kaunia lasiopthalma G.* The fractional separation was done using partition chromatography, HPLC and other techniques were utilized to separate different isolates. After the NMR spectral data and mass spectrometry (high resolution) the known compounds were haageanolide, 1–epi–reynosin, 1β–hydroxyarbusculin A, santamarin, santamarin acetate, reynosin, 4–epi-1β–hydroxyarbusculin A, 11,13–didehydrovulgarin, 3α,4α–epoxy-2α–isobutyryloxykauniolide, dehydroleucodin,jaceosidin, 2β–acetoxy-3α,4α–epoxy–3,4–dihydrokaunolide, 3–chlorodehydroleucodin, hispidulin, baynol C, eupafolinhispidulin and costunolide.

Out of the two novel compounds the first was obtained as a colorless gum. HPLC (HRESIMS) indicated the molecular formula to be $C_{17}H_{25}O_5$ with 6 degrees unsaturation. The infrared spectrum showed the presence of hydroxyl, ester and α, β–unsaturated–γ–lacxtone group. The NMR technique including COSY, HMBC, HMQC and NOESY spectra further clarified the structure of the compound. The data revealed that this compound was related to 4–epi–1β–hydroxyarbusculin with only difference that the hydroxyl group is replaced by acetyl group with C–1 inversed configuration and was thus named as 4–epi-1α–acetoxyarbusculin A. The structure of the compound is shown in Figure 12.

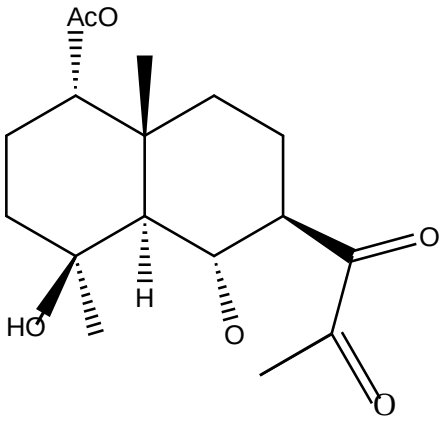


Figure 12: structure of 4–epi-1α–acetoxyarbusculin A.

The Second Novel compound was separated as clear oil and the HRESIMS (HPLC) characterization showed its molecular formula to be $C_{17}H_{22}O_4$, with 7 degrees unsaturation. The infrared spectrum confirmed presence of α,β–unsaturated–γ–lactone and an ester group. The NMR data further clarified the structure that the new compound was similar to acetate of reynosin. Thus, after data analysis the compound was identified as acetyl–1–epi reynosin and the structure of this compound is shown in Figure 13.

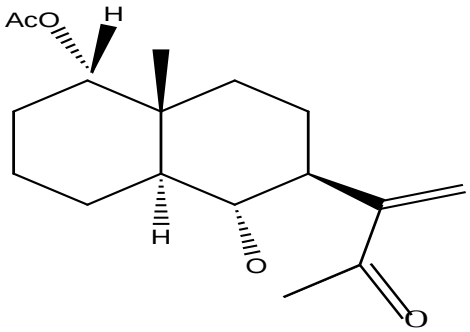


Figure 13: Structure of acetyl–1–epi reynosin.

Further, all the isolated sequiterpenes were later checked for their medicinal value and were found to be that they all have anti-Carcinogenic activities. Their activity was assessed on breast cancer cells and it was found that all the compounds (sesquiterpene lactones) had potent anti-Carcinogenic activity. Among the terpenes Custunolide was the most potent and only one compound methyl cysteine adduct was non-active on cancer cells.

Maldonado et al. (2013) in another research has worked on the isolation of terpenoids from *Kaunia lasiopthalma Griesb*, used plant's floral parts for this purpose. The plant gave a large number of sesquiterpenes that were known earlier and along with these a novel triterpene was also isolated. This triterpenoid was unusual from this plant and was found to cytotoxic. The structure of the compound was analyzed using NMR spectroscopy and the fractions were separated using chromatographic techniques. During the investigations for plant metabolites, it was observed to have unusually given a triterpenoid. This triterpene on characterization was found to be a novel compound with a different and unique carbon skeleton. It contained two α–methylene–γ–lactone moieties. During the separation, the compound was separated as clear oil and the HPLC (HRESIMS) revealed its molecular formula to be $C_{30}H_{38}O_7$. The ^{13}C and ^{1}H NMR data further clarified the structure of this novel compound having 30 carbon signals. Further, it indicated the presence of a ketone, an unsaturated double bond and α–β–unsaturated–γ–lactone. The compound on the basis of the structure was thus named as Kunial. This compound and the other sesquiterpenes were checked for their biological assay and their anti–carcinogenic activity. Kunial showed high cytotoxicity over the five-layer breast cancer cells and for this purpose the concentration was used in the range of 0.67 to 7.0 µM. The structure was thus characterized using NMR

techniques such as NOESY, HMBC, HSQC and ^{13}C NMR and the structure illustrated is shown in Figure 14.

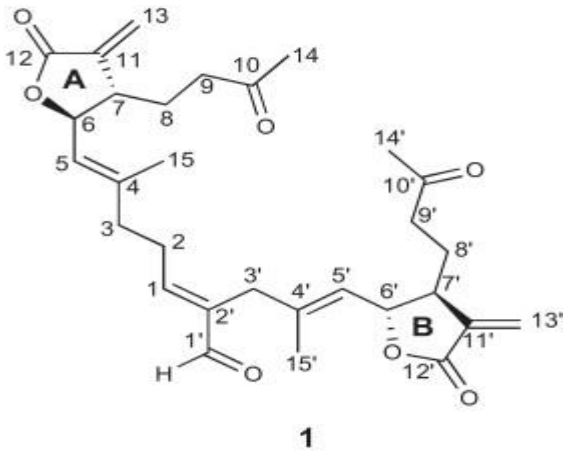


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Figure 14: Structure of the Novel Terpenoid (KAUNIAL).

Kim et al. (2018) has worked on the characterization of terpenoids from *Abies holophylla* of family Pinaceae. This plant was found to be home to a number of biologically active compounds including flavonoids, phenols, sterols, lignans and terpenoids. This plant possesses medicinal qualities as anti-hypertensive, anti-microbial, antiulcerogenic, anti-inflammatory, and as well as used for central nervous system. The extraction was done, and it was reported that there were seventeen lignans, three known triterpenoids and thirty diterpenoids. Out of the diterpenoids the other were known earlier and only three of them were found to be novel. Whereas, the triterpenoids that were three in numbers and were all novel. The structures of these compounds along with novel compounds were characterized using NMR (NOESY, ROESY, DEPT, COSY, HMBC, HSQC and HRMS) data. The mass spectrometric techniques were also utilized for the proper identification of the compounds.

The first Novel compound isolated was named as Holophyllin O (12 α–15–epoxy–13 β–hydroxyabiet–8 (14)–en–18–oicacid, which was separated as colorless gum with a molecular formula $C_{20}H_{30}O_4$. The second novel compound showed the formula (12β, 13E)– labdene–8, 12, 15–triol having a molecular formula $C_{20}H_{36}O_4$. Similarly, the third novel compound was a diterpenoid was named as Hallophyllin P (12 Z, 14 S)–12–labdene–8, 14, 15–triol) having a molecular formula $C_{20}H_{36}O_3$. The fourth novel compound was named as Holophyllane C with structural elucidation (25R)–3,4, seco–17, 14–friedo–23–oxo- 9β–lansota–4 (28), 6, 8 (14)–triene–3, 26–dioic acid. The fifth novel compound that was reported showed the molecular formula as $C_{30}H_{42}O_5$ ans was named as Hallophyllane D. The structural elucidation for this compound is 3,4, seco–8(14, 13R)-abeo–17, 13–friedo–23– oxo-9β H–lansota–4 (28) 7, 14 (30), 25 (27)–tetraene–3, 26–dioic acid. The sixth and last novel compound was Hallophyllane E having molecular formula $C_3H_{46}O_4$ and had planar structure. The structural elucidation showed only minor difference from Hallophyllane D as it had no carboxylic group instead was on olid and oate group at the end of the compound. All of the compounds along with novel di and triterpenoids were tested for their cytotoxicity against four human cancer cell lines (small cell lung adenocarcinoma, skin melanoma, malignant ascites and colon adenocarcinoma) and all were found to be active against the carcinogenic cells and Hollophyllane E showed high activity. Apart from this, these compounds were also checked for their activity as neuropathic, anti- malarial, anti–inflammatory and inhibition activity and more or less all the compounds showed some effects of medicinal grade. The structure of the six novel compounds is shown in Figure 15.

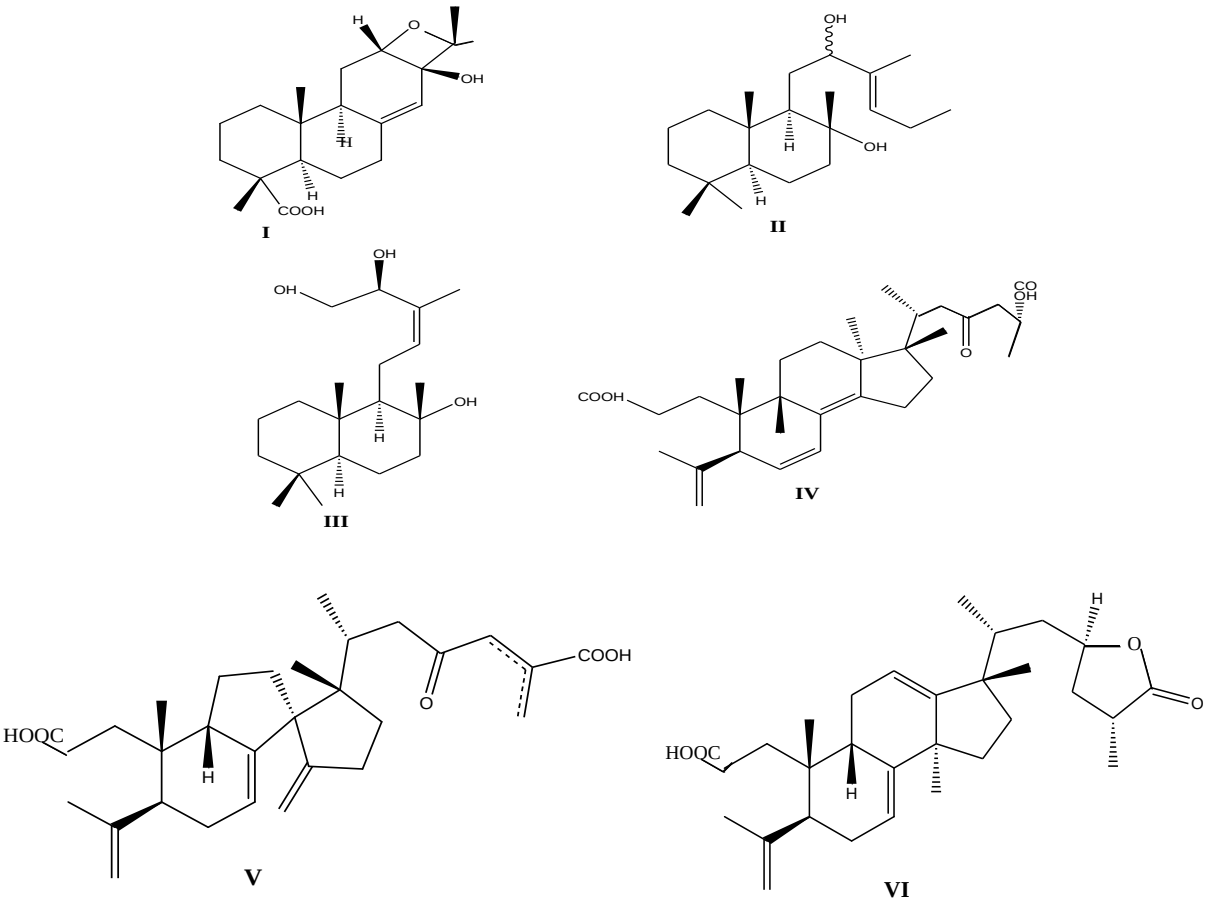

Figure 15: Structures of the six novel compounds from *Abies holophylla.*

CONCLUSIONS:

In this review article, eleven research articles related to the extraction, isolations and characterization of novel terpenoids from different plants and from different areas of the world have been thoroughly reviewed. The review further focuses on the different techniques which have been used during the extraction and isolation of these terpenoids from different parts of plants i.e. leaves, stem, roots and other vegetal parts of plants. During the study and reviewing of these research articles, it was found that various spectroscopic techniques such as IR, MS, NMR, etc., have employed for the structural characterizations of these terpenoids. The extracted and characterized compounds were found as terpenoids, triterpenoids, nortriterpenoids, monoterpenes, sesquiterpenes and macro cyclic monoterpenes. These compounds have been checked for their medicinal activity, cytotoxicity and their bioassay were also considered, where these compounds showed that they can be used as medicinal compounds as well as the bioassay confirmed their role as protective agents of the plants. The study of this review provides easy and comprehensive materials about the terpenoid present in plants, their extractions procedure, characterization techniques and uses as medicinal drugs against many diseases. Similarly, it was observed that there is a lot of space for the research in the field of isolation of different terpenoids from the plants and their activity and it is a fact that there are still compounds that are yet not explored.

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