

## Research Paper

# Phytochemical components and antioxidant properties of *Viscum album* growing on *Populus alba* host tree

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## Abstract

*Viscum album* is the parasitic medicinal plant with multiple pharmacological activities belonging to family Loranthaceae. The plant is widely used as in adjunct in cancer chemotherapy in Europe and has other pharmacological properties like antiviral, antidiabetic, anti-inflammatory and antihypertensive. The present studies involves identification and characterisation of the phytochemical constituents from the hexane and methanolic extract of the plant by GC-MS analysis and determination of antioxidant potential of the methanolic extract of the plant from *Populus alba* host tree. Twenty seven and thirty five chemical constituents were identified from the hexane and methanolic extract respectively. The major constituents from the hexane extract are pentacosane, (6Z, 9Z)-cis-3,4-epoxy-nonadecadiene, palmitic acid,  $\beta$ -amyrin acetate, lupeol acetate, heneicosane and phytol. The major constituents from the methanolic extract include 2,3-Di-O-methyl-D-xylopyranose, monomethyl inositol, 4-O-methylmannose, 4-trichloroacetyloxyimino-2-carene. In addition to that the methanolic extract was subjected to antioxidant evaluation by DPPH and hydroxyl radical scavenging activity. The study provides scientific evidence that the methanolic extract of *Viscum album* from *Populus alba* host tree have strong antioxidant properties.

**Keywords:** *Viscum album*, *Populus alba*, GC-MS, antioxidant, phytocomponents, Himalayan region.

## Introduction

Medicinal plants are considered to be the source for most of compounds of therapeutic value and even today's most of the drugs are plant derived natural products or their derivatives<sup>1,2</sup>. Medicinal plants used in the traditional medicine against cancer and diabetes can serve as useful source for the discovery of safer drugs to be used against diabetes and cancer<sup>3,4</sup>.

*Viscum album* (Loranthaceae) is a semiparasitic traditional medicinal plant growing on deciduous tree hosts and is distributed throughout Europe, Africa, Asia and Japan<sup>5</sup>. In Himalayas, *Viscum album* mostly grows from Kashmir to Nepal at an elevation of 12,000 to 27,000 m<sup>6</sup>. *V. album* has been used over the years in traditional medicine for the treatment of anxiety, insomnia, atherosclerosis, hypertension, diabetes, hypertension, and in complimentary cancer therapies<sup>7</sup>. Experimental investigations indicate the plant to show antiviral, antimyobacterial, anticancer, apoptosis-inducing and immunomodulatory activities<sup>8</sup>. The leaves and the twigs of the plant are reported to possess a number of active metabolites and number of pharmacologically active metabolite have been isolated from this plant belonging to triterpenes, phenolics, flavonoids, phenylpropanoides, diarylheptanoides, carbohydrates, fatty acids, viscotoxins, and lactins<sup>9</sup>.

The antioxidant properties of some plant metabolites are responsible for the medicinal and therapeutic value of several medicinal plants used in traditional medicine. Antioxidants have wide range of applications like food preservation, due to their ability of stop oxidative degradation of lipids. These factors have accelerated the screening of plants for possible medicinal and antioxidant properties, the separation and characterisation of diverse phytochemical and the development and usage of antioxidants of natural origin<sup>10,11</sup>.

In case of parasitic plants it has been observed that exchange of metabolites takes place between host tree and the parasitic plant<sup>12</sup>. The phytochemical profile and antioxidant potential of the plant is dependent on the host tree<sup>13</sup>. From literature survey, no published literature exists about the volatile chemical constituents of *V. album* growing on *Populus alba* host tree.

Therefore, the present investigation was taken to analyse the qualitative profile of volatile metabolites from the hexane and methanolic extract of the *V. album* growing on *Populus alba* host tree using GC-MS technique and to evaluate the antioxidant profile of the methanolic extract by DPPH method. Determining the full phytochemical qualitative composition of the volatile metabolites together with the knowledge of its antioxidant potential will help in interpretation of the multiple pharmacological responses of the plant.

## Materials and Methods

The plants of *Viscum album* parasitic on *Populus alba* host tree were collected from Budgam district of Kashmir valley. Authentication of the plant was done by Curator Centre for Biodiversity and Taxonomy, University of Kashmir and voucher specimen has been deposited in the Herbarium of Kashmir University. The plant materials was washed thoroughly with tap water, followed by distilled water and were shade dried. The material was powdered using blender and kept separately in air tight container until required for extraction procedure.

### Extraction

A portion of the plant material (15g) were weighed and homogenised with 100 ml of the extraction solvent hexane using mixer grinder, for about 1 hr. The mixture was centrifuged for ten minutes and the supernatants were filtered through watmann no.1 filter paper. The solvent was dried using rotary evaporator. The marc was again extracted with methanol by the same method as described for preparing hexane extract. The hexane and methanolic extracts obtained was stored in refrigerator till used for further studies.

### GC-MS analysis

GC-MS analysis was carried out with GC-MS QP2010 Plus, Shimadzu, Japan fitted with programmable head space auto sampler, auto injector and mass selective detector. Separation was carried out on RTX 5 MS (30 metre) capillary column with helium as carrier gas at a flow rate of 1.21 ml/min and a linear flow velocity of 40.1 cm/s. The column was held initially at 60° C for 4 min, then increased to 280°C with heating ramp 10°C/min and hold on time 24 min. the split ratio was (10:0). Injection temperature was 280°C; pressure was established at 73.3 kPa. MS parameters were: scan rate (m/z): 40-700amu under the electron impact (EI) ionisation (70 eV). The conformation of the identity of the compound was done by comparison of retention times and mass spectral fragmentation patterns with those data provided in Wiley and National Institute of Standards and Technology (NIST) libraries. Identification was achieved when a good match of mass spectrum was confirmed. The relative percentage of each constituent was calculated by comparing its average peak area to the total area.

### DPPH free radical scavenging activity

The radical scavenging activity of methanolic extract of *V. album* growing on *Populus alba* host tree was evaluated by DPPH method as described by Brand Williams<sup>[14]</sup> with some slight modifications. A stock solution of the extract was prepared in methanol to a concentration of 1mg/ml. Different volumes (10-100µl) of the stock solutions were transferred to test tubes and volume was adjusted to 1 ml using methanol to give solutions of extract with concentration range of 10-100µg/ml. 50µl each of the different concentration of plant extract were added separately to 2.5ml of DPPH solutions (.005%) taken in test tubes. 2.5µl of DPPH and 50 µl of methanol was taken as control solution. Ascorbic acid and BHT were used as positive control and were prepared in the same way as test solutions. The mixtures were vortexed and kept standing in dark at room temperature for 1 hr. Absorbance of

spectrophotometer was set to zero using methanol at 517nm. The absorbance of the resulting mixtures was recorded. The percentage inhibition of the mixture was determined by using the equation:

$$\text{Percent inhibition} = \left( \frac{Ac - At}{Ac} \right) \times 100$$

Where Ac and At are absorbance of control and test sample respectively. The experiments were taken in triplicate and average values were taken. The IC<sub>50</sub> values i.e., concentration of the sample required to scavenge 50% of DPPH free radicals was calculated by linear regression.

## Results and Discussions

The GC-MS analysis of the hexane extract revealed the occurrence of 39 peaks (Figure 1) of which 27 chemical compounds were identified (Table 1) accounting for 76.57% of the total. The main group of compounds present in hexane extract are hydrocarbons (34.35%), terpenes (14.42%), tocopherols (2.33%), ketones (1.13%), aldehydes (0.58%), epoxides (9.04%), esters (1.45%), carboxylic acid (8.74%), phytosterols (1.45%) alcohol (2.54%). The main constituents identified in hexane extract are pentacosane (27.01%), (6Z,9Z)-cis-3,4-epoxy-nonadecadiene (9.04), palmitic acid (8.74%), β-amyrin acetate (6.27%), lupeol acetate (5.71%), heneicosane (3.76%) and phytol (2.54%).

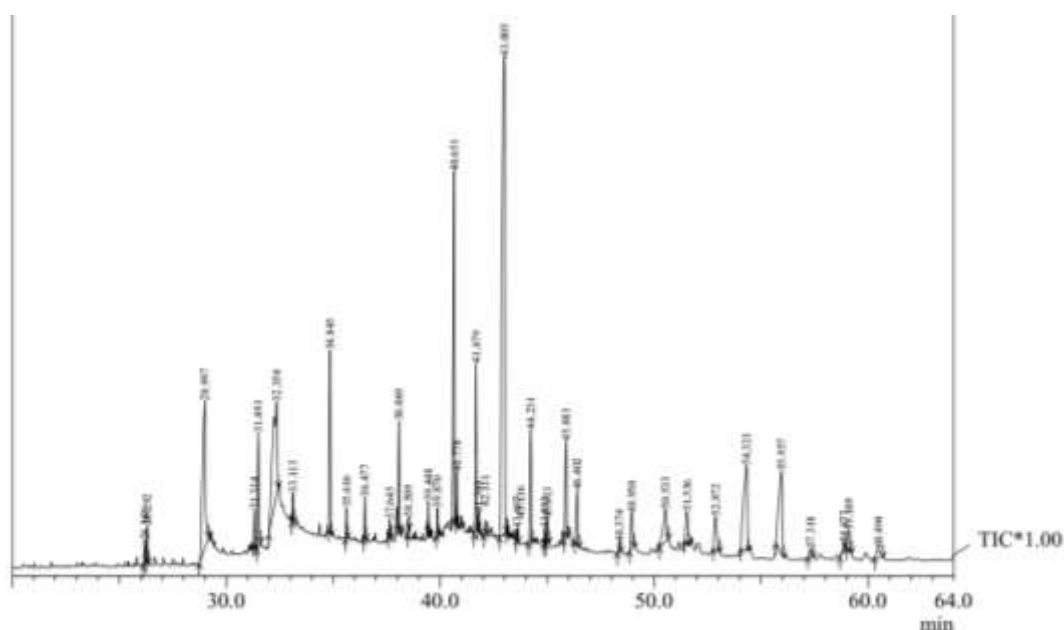


Figure 1: Typical GC-MS chromatogram of the constituents of hexane extract of *Viscum album* growing on *Populus alba* host tree

Table 1: Chemical composition of hexane extract of *Viscum album* growing on *Populus alba* host tree

Compound <sup>a</sup>	Molecular Formula	Molecular mass	R <sub>t</sub> <sup>b</sup>	Area (%)
Neophytadiene	C <sub>20</sub> H <sub>38</sub>	278	26.186	0.40
Hexahydrofarnesyl acetone	C <sub>18</sub> H <sub>36</sub> O	268	26.292	0.61
Palmitic acid	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>	256	28.997	8.74
Hexadecane	C <sub>16</sub> H <sub>34</sub>	226	31.314	0.62
Phytol	C <sub>20</sub> H <sub>40</sub> O	296	31.493	2.54
(6Z,9Z)-Cis-3,4-Epoxy-nonadecadiene	C <sub>19</sub> H <sub>34</sub> O	278	32.354	9.04
Heneicosane	C <sub>21</sub> H <sub>44</sub>	296	34.845	3.76
4,8,12,16-Tetramethylheptadecan-4-olide	C <sub>21</sub> H <sub>40</sub> O <sub>2</sub>	324	35.616	0.54
Undec-10-ynoic acid, tetradecylester	C <sub>25</sub> H <sub>46</sub> O <sub>2</sub>	378	37.645	0.34
Triacosane	C <sub>30</sub> H <sub>62</sub>	422	38.069	1.96
Bis (2-ethylhexyl)phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	38.509	0.21
2,6,10,14-tetramethylhexadecane	C <sub>20</sub> H <sub>42</sub>	282	39.448	0.32
Squalene	C <sub>30</sub> H <sub>50</sub>	410	41.799	0.28

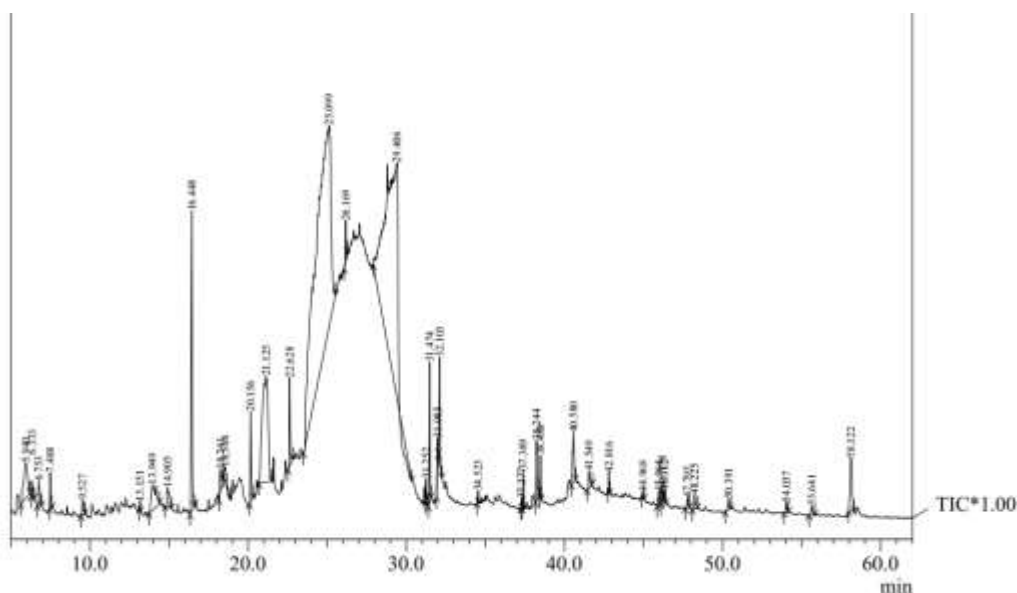
Compound <sup>a</sup>	Molecular Formula	Molecular mass	R <sub>t</sub> <sup>b</sup>	Area (%)
Hexacosanal	C <sub>26</sub> H <sub>52</sub> O	380	42.111	0.30
Pentacosane	C <sub>25</sub> H <sub>52</sub>	332	43.005	27.01
2-Nonadecanone	C <sub>15</sub> H <sub>30</sub> O	226	43.607	0.21
Heptacosanal	C <sub>27</sub> H <sub>54</sub> O	394	44.883	0.28
γ-Tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416	45.013	0.58
Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	46.402	1.75
2-Pentadecanone	C <sub>15</sub> H <sub>30</sub> O	226	48.374	0.31
(22E)-Stigmasta-5,22-dien-3β-ol	C <sub>29</sub> H <sub>48</sub> O	412	48.950	1.45
β-Amyrin	C <sub>30</sub> H <sub>50</sub> O	426	51.536	1.34
β-Amyrin acetate	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468	54.321	6.27
Lupeol acetate	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468	55.957	5.71
Methylcommate-C	C <sub>31</sub> H <sub>50</sub> O <sub>4</sub>	486	58.877	0.38
Phytyltetradecanoate	C <sub>34</sub> H <sub>66</sub> O <sub>2</sub>	506	59.109	0.90
Lupeol	C <sub>30</sub> H <sub>50</sub> O	426	60.494	0.72
Total identified %				76.57

<sup>a</sup>Compounds are listed in order of elution from the Rtx-5 column.

<sup>b</sup>Retention time (minutes).

NI: not identified

GC-MS analysis of methanolic extract of *V. album* growing on *Populus alba* led to the occurrence of 39 peaks in total ion chromatogram (Figure 2) from which 36 compounds were identified (Table 2), accounting for 99.48% of total. The main groups of identified compounds were namely sugars (48.51%), inositols (30.7%), alcohols (5.07%), ketones (2.38%), phenols (1.95%), terpenes (1.87%), carboxylic acids (1.45%), ester (1.28%), tocopherols (0.14%), hydrocarbons (0.57%), flavones (0.43%), aldehydes (1.01%), oxime ester (3.90%). The major phytochemical constituents identified from the methanolic extract include 2,3-Di-O-methyl-D-xylopyranose (41.07%), monomethyl inositol (30.71%), 4-O-methylmannose (7.44%), 4-trichloroacetyloxyimino-2-carene (3.90%). The distribution pattern of major groups of chemical constituents is shown in component bar diagram (Figure 3). The structure of some major phytocomponents is shown in (Figure 4).



**Figure 2: Typical GC-MS chromatogram of the constituents of methanolic extract of *Viscum album* growing on *Populus alba* host tree**

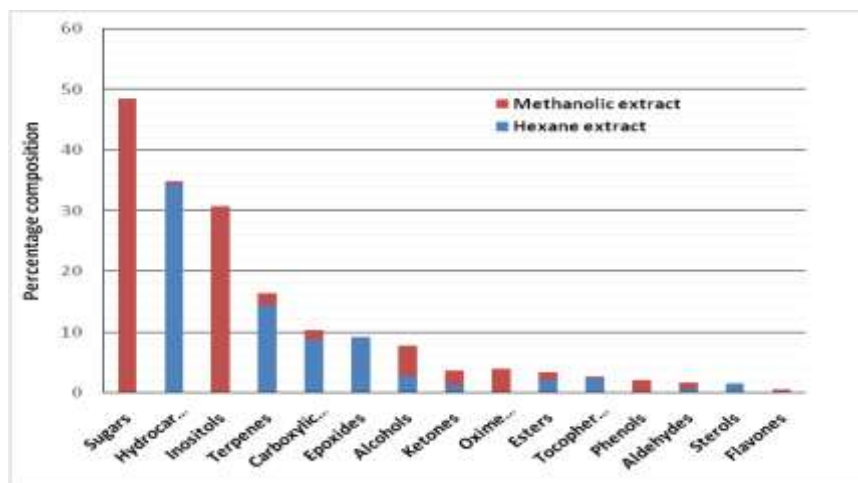
**Table 2: Chemical composition of methanolic extract of *Viscum album* growing on *Populus alba* host tree**

Compound <sup>a</sup>	Molecular formula	Molecular mass	R <sub>t</sub> <sup>b</sup>	Area (%)
1,2,3-Propanetriol	C <sub>3</sub> H <sub>8</sub> O <sub>3</sub>	92	5.940	1.73
1-(1'-pyrrolidinyl)-2-propanone	C <sub>7</sub> H <sub>13</sub> NO	127	6.333	0.21
1-Methylpyrrolidin-2-one	C <sub>5</sub> H <sub>9</sub> NO	99	6.753	0.47
Cyclohexane-1,2-diol	C <sub>6</sub> H <sub>12</sub> O <sub>2</sub>	116	7.488	0.36
3,5-Dihydroxy-6-methyl-2,3-dihydro-4H-pyran-4-one	C <sub>6</sub> H <sub>8</sub> O <sub>4</sub>	144	9.527	0.29
Bicyclo[2.2.1]heptan-2-ol,1,7,7-trimethyl, acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	196	13.151	0.05
Hydroquinone	C <sub>6</sub> H <sub>6</sub> O <sub>2</sub>	110	13.949	1.41
2,6-Dimethoxyphenol	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	154	14.905	0.56
4-Trichloroacetyloxyimino-2-carene	C <sub>12</sub> H <sub>14</sub> C <sub>13</sub> NO <sub>2</sub>	309	16.448	3.90
3,5-Dimethoxyphenol	C <sub>8</sub> H <sub>10</sub> O <sub>3</sub>	154	18.241	0.41
2-(Hydroxymethyl)-2-nitro-1,3-propanediol,	C <sub>4</sub> H <sub>9</sub> NO <sub>5</sub>	151	18.544	0.45
4-Methyl-2,5-dimethoxybenzaldehyde	C <sub>10</sub> H <sub>12</sub> O <sub>3</sub>	180	20.156	1.01
4-O-Methylmannose	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	21.125	7.44
Xanthoxylin	C <sub>10</sub> H <sub>12</sub> O <sub>4</sub>	196	22.628	0.98
2,3-Di-O-methyl-D-xylopyranose	C <sub>7</sub> H <sub>14</sub> O <sub>5</sub>	178	25.099	41.07
Neophytadiene	C <sub>20</sub> H <sub>38</sub>	278	26.169	0.27
Monomethyl inositol	C <sub>7</sub> H <sub>14</sub> O <sub>6</sub>	194	29.406	30.71
Methyl linolenate	C <sub>19</sub> H <sub>32</sub> O <sub>2</sub>	292	31.252	0.22
Phytol	C <sub>20</sub> H <sub>40</sub> O	296	31.474	1.27
Cis,cis Linoleic acid	C <sub>18</sub> H <sub>32</sub> O <sub>2</sub>	280	31.985	0.39
α.-Linolenic acid	C <sub>18</sub> H <sub>30</sub> O <sub>2</sub>	278	32.103	1.06
2-(Dimethylamino)ethyl-3-cyclopentylpropanoate	C <sub>12</sub> H <sub>23</sub> NO <sub>2</sub>	213	34.523	0.09
Palmitic acid-β-monoglyceride	C <sub>19</sub> H <sub>38</sub> O <sub>4</sub>	330	38.244	0.61
Bis(2-ethylhexyl)phthalate	C <sub>24</sub> H <sub>38</sub> O <sub>4</sub>	390	38.488	0.31
(E,E,Z)-1,3,12-nonadecatriene-5,14-diol	C <sub>19</sub> H <sub>34</sub> O <sub>2</sub>	294	40.580	0.98
4',5-Dihydroxy-7-methoxyflavone	C <sub>16</sub> H <sub>14</sub> O <sub>5</sub>	286	41.549	0.28
Tetracontane	C <sub>40</sub> H <sub>82</sub>	562	42.816	0.14
γ-Tocopherol	C <sub>28</sub> H <sub>48</sub> O <sub>2</sub>	416	44.968	0.06
Spiro(9,9'-bis(2-methyl-9H-fluorene))	C <sub>27</sub> H <sub>20</sub>	344	45.964	0.16
3',4',5-trihydroxy-3,7-dimethoxy-flavone	C <sub>17</sub> H <sub>14</sub> O <sub>7</sub>	330	46.178	0.15
Vitamin E	C <sub>29</sub> H <sub>50</sub> O <sub>2</sub>	430	46.312	0.08
Dehydrodiconiferyl alcohol	C <sub>10</sub> H <sub>14</sub> O <sub>3</sub>	182	48.225	0.28
Stigmast-5-en-3β-ol	C <sub>29</sub> H <sub>50</sub> O	414	50.391	0.23
24-Noroleana-3,12-diene	C <sub>29</sub> H <sub>46</sub>	394	54.037	0.21
Lupeol acetate	C <sub>32</sub> H <sub>52</sub> O <sub>2</sub>	468	55.641	0.23
2β,3β,23-Trihydroxyolean-12-en-28-oic acid	C <sub>31</sub> H <sub>50</sub> O <sub>5</sub>	502	58.122	1.43
Total identified %				99.48

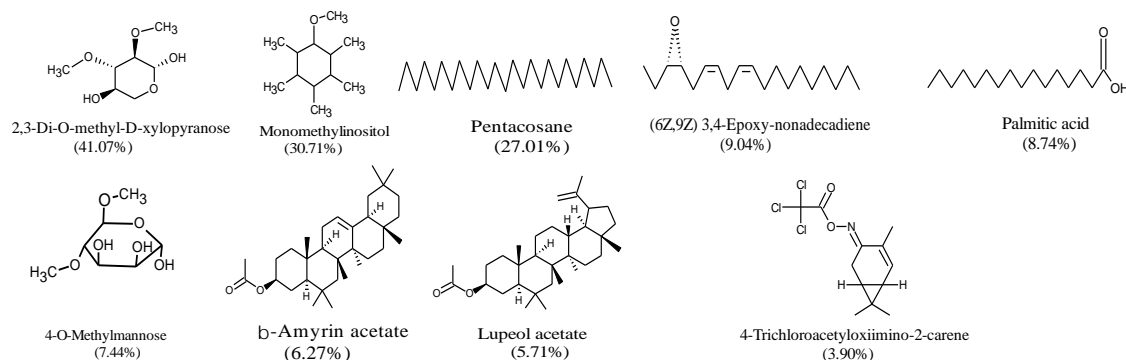
Some important phytoconstituents like lupeol acetate, vitamin E γ-tocopherol, phytol, neophytadiene and tetracontane and bis (2-ethylhexylphthalate) are found in both hexane and methanolic extract. The hexane (lypophilic) extract of *Viscum album* is extremely sticky called as viscin and is used in tropical treatment of ulcers, eczema, granulating wounds and burns<sup>15</sup>. Previous phytochemical investigations on the hexane extract has revealed the presence of different triterpenoids like bitulinic acid, ursolic acid<sup>16</sup> and olealonic acid<sup>17</sup>. Our present investigations on the hexane extract of the *V. album* growing on *Populous alba* host tree involves the presence of 14.42% of triterpenes, including β-amyirin, lupeol acetate, β-amyirin acetate, methylcommate C. and lupeol.

Tocopherol has a preventive effect during conditions of oxidative stress. γ-tocopherol is helpful for suppressing of melanogenesis and mRNA expression of tyrosinase and tyrosinase related protein-2 in B16 melanoma cells<sup>18</sup>. Stigmast-5,22-dien-3-ol (stigmasterol) is an anticancerous phytosterol. Stigmast-5-en-3β-ol (β- Sitosterol), is an anti-inflammatory phytosterol and shows anti-arthritis, anti-

pyretic, estrogenic and insulin releasing effects<sup>19</sup>. The triterpenes are important class of natural products proven to show a wide spectrum of biological effects like anti-bacterial, anti-viral, anti-fungal, anti-oxidative and anti-inflammatory properties<sup>20</sup>. Lupeol is reported to be active against inflammation, tumorous growths, protozoa<sup>21</sup>.



**Figure 3: Percentage of major groups of chemical constituents in the methanolic and hexane extract of *Viscum album* growing on *Populus alba* host tree**

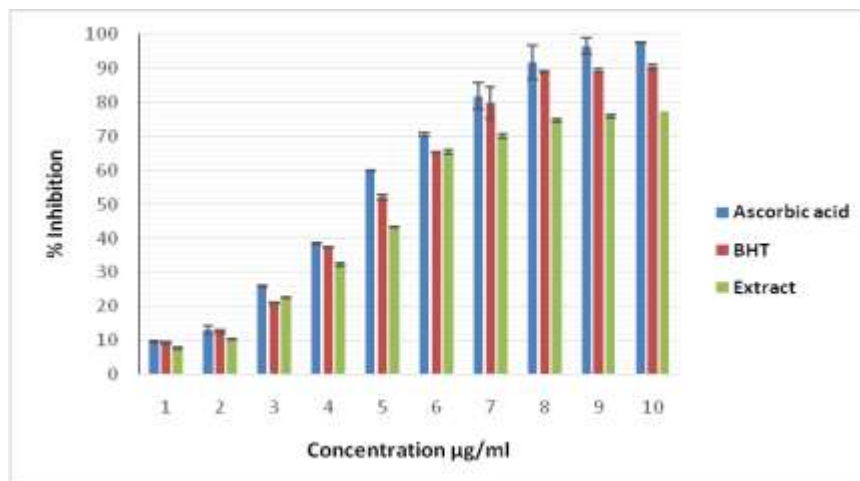


**Figure 4: Main Components of the major groups of chemical constituents in the methanolic and hexane extract of *Viscum album* growing on *Populus alba* host tree in Kashmir Himalayan region**

Monomethyl inositols is found to be present in the methanolic extract to the extent of 30.7%. Previous studies from this plant has revealed the presence of D-Pinnitol (1-D-3-O-methylchiro-inositol) and L-quebrachetol (1-L-2-O-methylchiro-inositol) in the leaves and stem of this plant irrespective of the composition of host tree<sup>22</sup>. 1-D-1O-methyl muco-inositol has also been reported from this plant<sup>23</sup>. Inositols are stress metabolites and protect the plant against environmental stresses like extreme heat, salt exposure, exposure to reactive oxygen species, hydrostatic pressures etc<sup>24</sup>. *V. album* experiences cold and drought stress during winters which induces the accumulation of mono-O-methyl-inositols in the plant<sup>25,26</sup>. Inositols in the plants have multipurpose functions acting as cryoprotectants, stress metabolites, carbon storage compounds and osmotica<sup>23</sup>. The role of inositols in diet has been established as treatment for Type-1 diabetes for improving the insulin mediated glycemic control<sup>27</sup>. Inositols play a role in the treatment of Polycystic ovarian syndrome<sup>24</sup>.

The antioxidants from plants have a good therapeutic potential for the treatment of oxidative stress related disorders<sup>28</sup>. The antioxidant potential of *V. album* has been found to depend on the harvest season and nature of the host tree. The evaluation of antioxidant activity of methanolic extract of *V. album* from different host trees<sup>13</sup>, shows that the plant from lime tree in summer shows highest DPPH and anti-lipid peroxidation activity. The DPPH percentage inhibition of the methanolic extract of *V.*

*album* growing on *Populus alba* host tree is shown in figure 5. The methanolic extract exhibited a concentration dependent antioxidant effect which was comparable to reference standards ascorbic acid and butylated hydroxytoluene at the same concentration. The IC<sub>50</sub> value of the extract was found to be 57.12±0.191µg/ml. The methanolic extract showed significant antioxidant activity compared to standard.



**Figure 5: Antioxidant activity measured by DPPH radical assay of methanolic extract of *Viscum album* hosted by *Populous alba* tree in Kashmir Himalayan region and compared with ascorbic acid and BHT.**

The present study shows that *V. album* growing on *populous alba* host tree has a radical scavenging activity. In our previous studies<sup>29</sup> the antioxidant activity and the volatile constituents of *V. album* from *Juglans regia* host tree were found to be different from our present studies. Thus our study clearly shows that *V. album* plant parasitic on different host trees, has different antioxidant and phytochemical profile.

### Conclusion

The present study shows that *V. album* growing on *Populous alba* is rich in volatile components associated with diverse pharmacological activities. The methanolic extract of the plant has significant antioxidant activity. The plant can be a potential source of new antioxidants and therapeutically valued metabolites against various diseases. However, further studies are needed to isolate and characterise the phytocomponents responsible for the activity.

### Acknowledgement

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