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Chemical composition and antioxidant activity of *Viscum album* L. growing on *Juglans regia* host tree in Kashmir, India

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ABSTRACT

In the present work, methanolic extracts of Viscum album young leaves and branches were evaluated, in order to identify the chemical constituents that confirm its biological activity. GC-MS technique was employed for separation and identification of the extracted components. The analysis revealed the presence of terpenoids, , sugars, inositols and natural antioxidants such as phenolic compounds and vitamin E. The antioxidant potential of the extract was also evaluated by DPPH method. The methanolic extract was observed to have a powerful antioxidant effect with an IC_{50} value of 61.76µg/ml.

Keywords- Antioxidant activity, DPPH, fatty acids GC-MS, Juglans regia, triterpenes, Viscum album,

I INTRODUCTION

Plants are one of the most important sources of medicines. Since the beginning of human civilization, medicinal plants have been used by mankind for their therapeutic value. The majority of new drugs have been produced from natural products (secondary metabolites) and the compounds derived from natural products [1].These invaluable products are explored for the potential discovery of novel biomolecules that have possible future uses. The traditional medicine system used in China[2] and India[3] are based on the use of medicinal plants. The use of plants in the traditional system of medicine of many other cultures has been extensively documented [3]. These plant based traditional systems of medicine continue to play an essential role in health care, and the World Health Organization has estimated that approximately 80% of the world's inhabitants rely mainly on traditional medicines for their primary health care [4].

Traditional medicines from plants have attracted major attention worldwide because of their potential pharmaceutical importance and are gaining popularity in the prevention and treatment of various diseases

Viscum album L. belongs to the family Loranthaceae[5] within the order Santalales. *Viscum. album* L. is an evergreen dioecious small parasitic shrub growing on a tree host in temperate Europe and western Asia. Three subspecies for this parasitic plant are recognised, Two subspecies grow on coniferous host, V.

album L. subsp. abietis (Wiesb.) Abrom. on fir (Abies Mill.), Viscum. album L. subsp. austriacum (Wiesb.) Vollm. on Scots pine (Pinus sylvestris L.), the third one is V. album L. subsp. album, growing on decidous trees, mostly on apple (Malus Mill.), poplar (Populus L.) and willow (Salix L.), but sometimes on oak (Quercus L.) and never on beech (Fagus L.) [6]. *Viscum album* is widely distributed in tropical and subtropical Africa, Asia and Europe [7]. The plant is widely distributed from central Nepal to Afganistan to temperate Eurasia at an altitude of 1000-2700m. The plant is commonly found on old walnut trees in Kashmir. Flowering takes place from march-may. Flowers yellowish- green, in stalkless clusters in the axil of the branches, with cup shaped ciliate bracts; perianth segment triangular, thick. Leaves variable, usually 3-5 cm long, thick fleshy; stems forked, 60-90 cm. berry globular white translucent[8].

Viscum album has been used for the treatment of many diseases, like as hypertension, diabetes, asthma, infertility, epilepsy, vertigo and lumbago for in traditional medicine [9]. A number of biological activities, such as anticancer [10] antimycobacterial [11], antioxidant[12] antiviral [13] apoptosis-inducing [14], cytotoxic [15] immunomodulatory[16], activities of mistletoe extracts have been observed.. Many herbal preparations containing mistletoe extracts (e.g. Iscadorr, Helixorr, Isorel) are also used in complementary cancer treatement [14].

As per the authors knowledge no studies have been carried out on the volatile chemical constituents of *Viscum album* growing on *Juglans regia* tree host from any part of the world.. Therefore, this study has been taken to carry out the chemical investigation of volatile chemical constituents of the important parasitic medicinal plant *Viscum album* growing on *Juglans regia (walnut)* host tree and to determine its antioxidant properties.

II MATERIALS AND METHODS

Experimental

Viscum album was collected in September 2015 from *Juglans regia* tree from Kashmir. The identity of the plant material was authenticated by the taxonomist at Centre for Biodiversity and Plant Taxonomy University of Kashmir. The plant material was washed by tap water followed by distilled water and was shade dried. The shade dried plant material was crushed using pestle mortar and kept in polethylen packets till further use.

The crushed plant material (30g) was homogenised with 200ml, methanol using a commercial blender for 1 hour. The marc was again extracted with methanol by the method as described for hexane. This mixture was centrifugated for 10 minutes and the supernatants were filtered through a whatman no. 1 filter paper. The filtrate was evaporated to dryness by rotary evaporator and weighed.

Mass spectrometry

Plant extracts were analysed on a GC-MS-QP2010 Plus (Shimadzu, Kyoto, Japan) system with head space sampler (AOC-20s) and auto injector (AOC-20i), equipped with mass selective detector,

Helium was used as the carrier gas , at a costant flow rate of 1.21 mL/min, and a linear flow velocity of 40.1 cm/s. Compounds were separated using a Rtx 5 MS capillary column (Restek Company, Bellefonte, USA: crossbond 5% diphenyl/ 95% dimethyl polysiloxane) having dimensions 30 m (length) × 0.25 mm (diameter) × 0.25 µm (film thickness). Column programme was set as following : 60°C

The ionization parameters were set as follows: ion source temperature of 230°C, interface temperature of 290°C, a solvent cut time of 4.50 min threshold of 1,000 eV and mass range of 40 to 700 m/z. the conformation of the identity of the compounds was done by comparison of their mass spectral fragmentation patterns with those data provided in WILEY8.Lib, NIST08.Lib, NIST08s.Lib and NIST.Lib. Identification was assumed when a good match of mass spectrum was achieved. The relative percentage area of each constituent was calculated by comparing its average peak area to the total area.

Antioxidant activity

The free radical scavenging activity of the methanolic extract *Viscum album* growing on *Juglans regia* tree host was evaluated by DPPH method using the known methodology[18] with some modification . Different concentrations of the antioxidant solution in methanol (50 µL) was added to 1.95 mL of a 0.005% DPPH solution in methanol. The solutions were incubated at 30°C for about 30 minutes and the decrease in absorbance was determined at 517 nm. The percentage inhibition were calculated by using the equation $[(A_0-A_1/A_0) \times 100]$ where A_0 was the absorbance of control and A_1 the absorbance of the plant extract/standard. ascorbic acid was used as positive control and DPPH and methanol (50 µL) in place of plant extract as negative control. All tests were run in triplicate and average values were calculated

III RESULTS AND DISCUSSION

The study on the volatile chemical constituents of *Viscum album* growing on *Juglans regia* tree host by GC-MS analysis exhibited the presence of 39 peaks in the methanolic extract (fig. 1), of which 35 chemical constituents were identified which are given in Table 1. The quantitative estimation of the chemical constituents of the plant studied showed that the methanolic extract of *Viscum album* is rich in terpenoids, phenolics sugars and inositols, esters and fatty acids. The major chemical constituents identified in the methanolic extract are 2,3-Di-O-methyl-D-xylopyranose(31.13%), glycerol(20.06%), 4-O methylmannose(5.13%) and two undifferentiated isomers of monomethylinositols(22.35%). Most of these the chemical constituents identified from this plant are reported to be pharmacologically very active compounds. The triterpene lupenone and its derivatives like lupeole acetate are reported to have different biological effects like antioxidative and anti-inflammatory effects[19] Lupenone is also found to possess strong inhibiting activities on the herpes simplex viruses HSV-1 and HSV-2 and African swine fever virus (ASFV) [20]. Linoleic acid identified in the plant is an essential fatty acid and its deficiency in diet causes mild skin scaling, hair loss[21], and poor wound healing in rats[22]. Linolenic acid an Omega-3 fatty acids help in the reduction of inflammation in the body and can also lower risk factors related to heart disease and arthritis. alpha-linolenic acid has beneficial effect on cardiovascular health and reduce risk factors for strokes, heart attacks, and high blood pressure. [23]

The methanolic extract of the *Viscum album* growing on *Juglans regia* showed a strong antioxidative effect which is expected mainly due to a number of phenolic components like flavonoids, phenolic acids, and phenolic diterpenes etc.[24] . The presence of tocopherol and vitamin E a natural antioxidant can also be responsible for high antioxidant character of *Viscum album* parasitic on *Juglans regia*.

These phenolic components possess many hydroxyl groups including o-dihydroxy group which have very strong radical scavenging effect and antioxidant power. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 1, 1-diphenyl-1, 2-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in methanol solution centred at 517 nm. The methanolic extract of of *Viscum album* showed antioxidant activity with IC50 values of and 42.5 respectively at 100 mg/mL concentration (Table 2). Free radical scavenging activity of methanolic extract) was confirmed in the present investigation (Fig. 2). Thus, the identification of a number of active chemical constituents like phenolis, Vitamin E, triterpenes etc supports the claim of the use of this plant in traditional medicine. The compounds responsible for this activity needs to be isolated and further investigated.

Table 1. Chemical composition of methanolic extract of *Viscum album* growing on *Juglans regia* (walnut) tree.

Compound Name	Molecular Formula	Retention time	relative %
Glycerol	C3H8O3	7.494	20.06
Glycerol-α-monoacetate	C5H10O4	8.292	2.47
Methyl-2-methylidene-5-oxohexanoate	C8H12O3	10.644	0.16
2,3-Dihydro benzofuran	C8H8O	11.916	0.38
2-Methoxy-4-vinylphenol	C9H10O2	13.953	1.06
2, 6-dimethoxyphenol	C8H10O3	14.877	1.39
unknown		15.865	1.04
3, 5-dimethoxyphenol	C8H10O3	18.175	2.22
1,6-anhydro-β-D-Glucopyranose	C6H10O5	19.699	1.22
4-Methyl-2,5-Dimethoxybenzaldehyde	C10H12O3	20.166	1.65
4-O-Methylmannose	C7H14O6	21.328	5.13
Megastigmatrienone 2	C13H18O	21.616	0.16
2,5,5-Trimethyl-4-methylene-1,2,3,4,5,6,7,8-octahydro-2-naphthol	C14H22O	22.374	0.34
Xanthoxylin	C10H12O	22.650	1.70
2,3-Di-O-methyl-D-xylopyranose	C7H14O5	24.568	31.15
Monomethylinositol*	C7H14O6	26.657	7.23
Monomethyl inositol*	C7H14O6	28.805	15.12
Methyl linoleate	C19H34O2	31.144	0.67
Methyl linolenate	C19H32O2	31.252	0.87
Phytol	C20H40O	31.473	0.26
Linolic acid	C18H32O2	31.979	0.49
α-Linolenic acid	C18H30O2	32.092	0.93
Ethyl linoleate	C20H36O2	32.354	0.25
Ethyl α-linolenate	C20H34O2	32.467	0.27
Phytol,acetate	C22H42O2	33.306	0.11
Heneicosane*	C21H44	34.806	0.12
Heneicosane*	C21H44	36.455	0.08

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Heneicosane*	C21H44	38.041	0.12
Glycerol–β–palmitate	C19H38O4	38.233	0.14
Bis(2-ethylhexyl)phthalate	C24H38O4	38.484	0.09
1-Iodo-dotriacontane	C32H65I	39.430	0.08
(Z)-7-Hexadecenal	C16H30O	40.581	0.18
α-Tocopherol	C28H48O2	44.958	0.12
Vitamin E	C29H50O2	46.311	0.22
Methylcommate D	C31H50O4	50.588	0.40
Lupenone	C30H48O	51.892	0.27
24-Noroleana-3,12-diene	C29H46	54.032	0.78
Lup-20(29)-en-3-yl acetate	C32H52O2	55.648	0.76
2β,3β,23-Trihydroxyolean-12-en-28-oic acid methyl ester	C31H50O5	58.070	0.32

* Isomers not differentiated

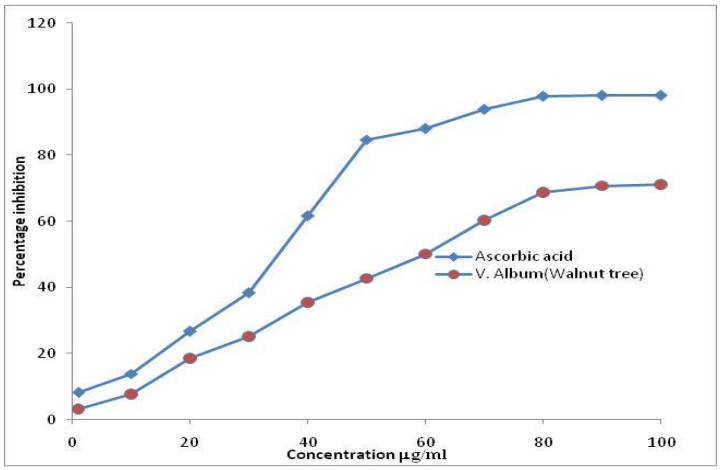

Fig. 2. DPPH antioxidant activity of Viscum album on Juglans regia host tree.

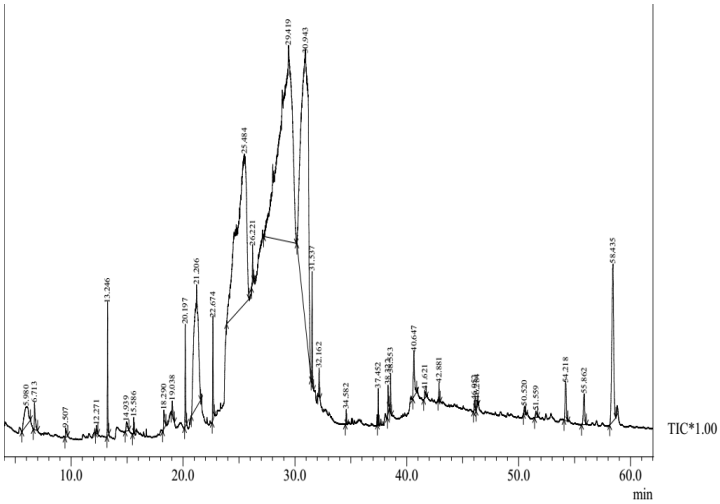

Fig. 1. TIC of methanolic extract of *Viscum album* on Juglans regia host tree.

IV CONCLUSION

The results of this study show that methanolic extract of V. album is rich in phenolic compounds having potential antioxidant properties and can be considered as good source for medicinal applications. Isolation of the metabolites from this plant and in-depth study of their biological activity will ensure best possible results in future and open new opportunity for discovery of potential drugs of therapeutic worth from this plant.

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