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EXPLORATION OF ANTIDIABETIC POTENTIAL OF TRADITIONAL ETHNO-MEDICINAL PLANT *VISCUM CRUCIATUM* SIEBER EX BOISS. (LORANTHACEAE) FROM RAWALAKOT DISTRICT, POONCH, AZAD JAMMU AND KASHMIR, PAKISTAN

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Abstract

This research aims to explore the antihyperglycemic potential of *Viscum cruciatum* Sieber ex Boiss., a locally known ethnomedicinal plant called "Parwikh" from the family Loranthaceae, found in Rawalakot, District Poonch, Azad Jammu and Kashmir (AJK), Pakistan. The plant has been traditionally used for the treatment of type-2 diabetes mellitus by local communities. Due to its hemiparasitic nature and association with host species *Quercus incana* and *Olea ferruginea*, it was hypothesized that the plant may possess unique medicinal properties derived from its host interactions for differential cure of diabetes. Traditional ethnomedicinal (TEM) knowledge was collected through Open and closed-ended questionnaires and microstatistical tools, such as informant consensus factor (ICF) and fidelity level (FL for data authentication. Preliminary phytochemical screening of leaf and bark extracts revealed the presence of flavonoids, saponins and alkaloids. For antihyperglycemic analysis, experiments were conducted on alloxan-induced Type-2 hyperglycemic rabbits using methanolic extracts of leaf and bark at concentrations of 500 mg/kg and 1000 mg/kg, administered orally for 7, 15 and 30 days. Blood sugar levels were measured using an ACCU-CHEK-active model glucometer. The results demonstrated that the rabbits treated with 1000 mg leaf extract from the host *O. ferruginea* exhibited the maximum decrease in blood glucose level (1491.5 mg/dL). The same treatment showed the highest decrease in body weight (25.36%), while the T3-group treated with 500 mg bark extract from the host *Q. incana* showed a less decrease in blood sugar (8.22%). The same treatment displayed the maximum increase in serum insulin level (5.790.11 pmol/L) on the 15th day of treatment. This study establishes the significant antihyperglycemic properties of the methanolic extract of *Viscum cruciatum* Sieber ex Boiss from host of *Olea ferruginea*, validating its traditional use in Rawalakot, AJK territory of Pakistan, for the treatment of type-2 diabetes mellitus. The findings suggest that further comprehensive research on this plant could lead to discovery and the development through optimization protocols as the novel allopathic drugs.

Key words: Traditional ethnomedicinal plant; *Viscum cruciatum* Sieber ex. Boiss.; Medicinal plant; Hypoglycemic; Ethnobotany.

Introduction

Human beings have been suffering from various infirmities since their emergence on this planet and plants have been employed to cure different acute and chronic diseases. The diseases oozing due to metabolic disorders and physiological changes are prevalent and out of these diabetes has been recognized as one of top ten deadly diseases. Many studies regarding diabetics have declared them killer diseases, as they are the root of many other diseases like heart disorders, kidney problems, blood pressure issues, and many more (Abinaya *et al*., 2020). It is a metabolic disorder caused by defects in insulin production, action, or both in humans (Waheeda *et al.*, 2016). The hyperglycemia disorder may accompany many other infirmities like food ulcers, nephropathy, neuropathy, hypertension, hyperlipidemia, retinopathy, cardiac issues, skin problems and eye-sight loss (Kumar *et al*., 2021; Waheeda *et al.*, 2016). Currently, type II diabetes mellitus (T2DM) is prevalent in the world and increasing very rapidly due to food contamination, sedentary lifestyles, environmental changes and various type stresses which may be due to the social or culture revolution. It is estimated that Type 2 diabetes mellitus (T2DM) will rise by 5.5-7.7% in the years 2025–2030 (Waheeda *et al.*, 2016). It will lead to the spread of many chronic diseases, leading to a health plethora and dilemma in the coming decades (Unnikrishnan *et al.,* 2017).

Diabetic conditions are caused by reduced insulin secretion or long-lasting glucose concentrations in the blood (Araki *et al.*, 2010; Kumar *et al*., 2021). Unrelenting persistence of hyperglycemia increases oxidation stress, which provides room for auto-oxidation of glucose and causes alterations in polyol pathway activity, accumulating free radicals in individuals and damaging cells that produce insulin in the body (Nirmala *et al.,* 2011). Sometimes, various self-defence systems of organisms (like macrophages, t-cells and killer cells) also damage t-cells. Malfunctioning of T-cells results in T2DM which not only increases blood sugar levels but also causes a number of complications and disorders in the body (Abinaya *et al*., 2020; Arora *et al*., 2021; Szurpnicka *et al*., 2019).

To treat diabetics, various techniques, medications and methods are used. Some of the most commonly used treatments are insulin injections, oral allopathic medicine stem cell therapy and islet transplantation. These techniques are not widely preferred, as giving insulin injections twice a day not only causes anxiety issues but also a financial problem owing to its high cost moreover allopathic medicines have several side effects on the body's physiology or stomach. Whereas islet transplantation is an expensive process and has recently been unavailable in Pakistan, there are also chances of tissue rejection by the body. In light of the above-mentioned issues, diabetic patients are looking forward to an appealing treatment with more advantages and lower health risks (Bussing, 2004). In respective aspects, herbal treatment has proven much more effective against various diseases (Roy *et al*., 2022).

Since the emergence of man on the planet, he has been utilizing various plants and their by-products for curing various acute and chronic diseases, believing plants to be *mystics of nature*. Afterwards, with the advent of medical science, herbalists and ethnobotanists discovered that every plant has a special combination of phytochemicals that are responsible for the '*mystic effects of the plant',* which is the real reason for the use of herbal drugs to cure diseases (Ishtiaq *et al.,* 2016). Worldwide, a number of different wild plants are used as sources of TEMs in different cultures around the world (Raghuvanshi *et al*., 2021). Owing to the presence of a wide variety of powerful phytochemicals in plants, many of these are extensively used to cure a number of diseases as herbal medications, which are named differently in various areas of the world (Roy *et al*., 2022). Wild medicinal plants are known as an ironic source for producing many allopathic and homeopathic medicines to cure various fatal ailments in humans and animals. It is estimated that nearly 50 to 60% of medicines are produced from plants and their products or extracts. It is now an established fact that allopathic medicines have many side effects, are costly, are difficult to access, which makes room for the renaissance of herbal therapeutics, which are cost-effective, better in treatment due to synergistic effects and easily available to indigenous people. Hence, people are coming towards the use of '*plant-based medicines'* to cure various diseases (Raghuvanshi *et al*., 2021; Ishtiaq *et al.*, 2021).

This proves that plant-based botanic drugs are safe, cost-effective and easy to access. The current research is an effort to provide some concrete bases for treatment of diabetes with the phytochemically rich plant *V. cruciatum.* It has multiple ethnomedicinal uses as it is used for treating various diseases like nausea, vomiting, cancer, diarrhoea, and hypertension. Owing to the versatile phytochemical composition of *V. cruciatum*, it is also used as a constituent of various herbal recipes (Nkem *et al.*, 2007). Since pre-historic times, people have believed in the magical powers of *V. cruciatum,* which is considered a traditional plant and used during Christmas celebrations. *V. cruciatum* is also often called the 'Christmas Plant, as cited in the literature (Nickrent *et al.*, 2010).

Viscm cruciatum Sieber ex Boiss. Belongs to family Loranthaceae and morphologically it is a hemiparasitic evergreen shrub of. The plants have a curious mode of nutrient and water absorption from host photosynthesis tissues in competitive mode and microbes are very high owing to nutrient scarcity; hence, they have a high concentration of offensive and defensive compounds (Ishtiaq *et al.,* 2013). In Azad Kashmir (Pakistan), two species, viz., *Viscum album* and *V. cruciatum* are reported (Waheeda *et al*., 2021). The former one bears white berries, while *V. cruciatum* bears red berries (Ishtiaq *et al.,* 2013). According to Nickrent *et al*., (2010), mistletoe the taxonomic ranks their systematic position are rather dubious and demand detailed taxonomic studies for their proper and authentic identification, satisfying quality control of herbal drugs from these plants (Ishtiaq *et al.*, 2017).

The key objectives of the present research were: (i) to document traditional ethnomedicine (TEM) uses of *Viscum cruciatum* Sieber ex Boiss. being prevalently used by indigenous communities the in-study area, (ii) to explore the hypoglycemic potential of *Viscum cruciatum* in treating type-2 diabetes, and (iii) to compare the effects from different host species (*Quercus incana* and *Olea ferruginea*) to explore potential differential effects on diabetes treatment with aim to verify its potential for allopathic drug discovery and development.

Material and Methods

Sample collection: Fresh and healthy leaf specimens of *Viscum cruciatum* Sieber ex Boiss. from hosts *Olea ferruginea* Royle and *Quercus incana* were collected during months of November and December in 2019 from the locality of Pearl valley, Rawalakot, Poonch, AJK. Collected specimens of *Viscum cruciatum* Sieber ex Boiss. identified, dried and mounted on herbarium sheet with proper voucher number (MUH-888) and deposited in herbarium of Department of Botany, MUST Mirpur (AJK) for future reference and research following method of Ishtiaq *et al*., (2013; 2017).

Traditional ethnomedicinal (TEMs) analysis: The data collection process involved the utilization of both semi-structured and structured interview protocols, following specific procedures that encompassed questionnaires and field plant interviews (Ishtiaq *et al*., 2013, 2021). To ensure a comprehensive approach to data gathering, we carefully planned field trips to the study area. Prior to conducting the data collection and ethnobotanical survey in Rawalakot, Poonch, AJK, we obtained proper authorization and permission from the relevant authorities (Ref No: 31/OCT/BOT/2019; Date: 21/10/2019). Additionally, an official field permit (Ref No: DFO/655/2019, Dated: 01/11/2019) was acquired from the District Forest Officer (DFO) of the Forest & Wildlife Department of Rawalakot to conduct field visits within the forest area of Rawalakot, Poonch, AJK.

TEM data was gathered through open and closed-ended interview protocols following methods (Ishtiaq *et al*., 2017; Maqbool *et al*., 2019; Ishtiaq *et al*., 2021). A questionnaire was designed and data was documented through random and planed field visits of different villages of Rawalakot, AJK (Fig. 1). Local translator and guide was hired who assisted in interview with indigenous communities and sampling sites identification to collect plants. For collection of traditional ethnomedicinal knowledge (TEK) of the plant, village heads were approached and consent was obtained to collect the data form local people and use this for thesis and research paper publication (keeping the identity of informants anonymous). The collected plants were identified with the help of Flora of Pakistan (Nasir, 1974; Abdullah, 1973).

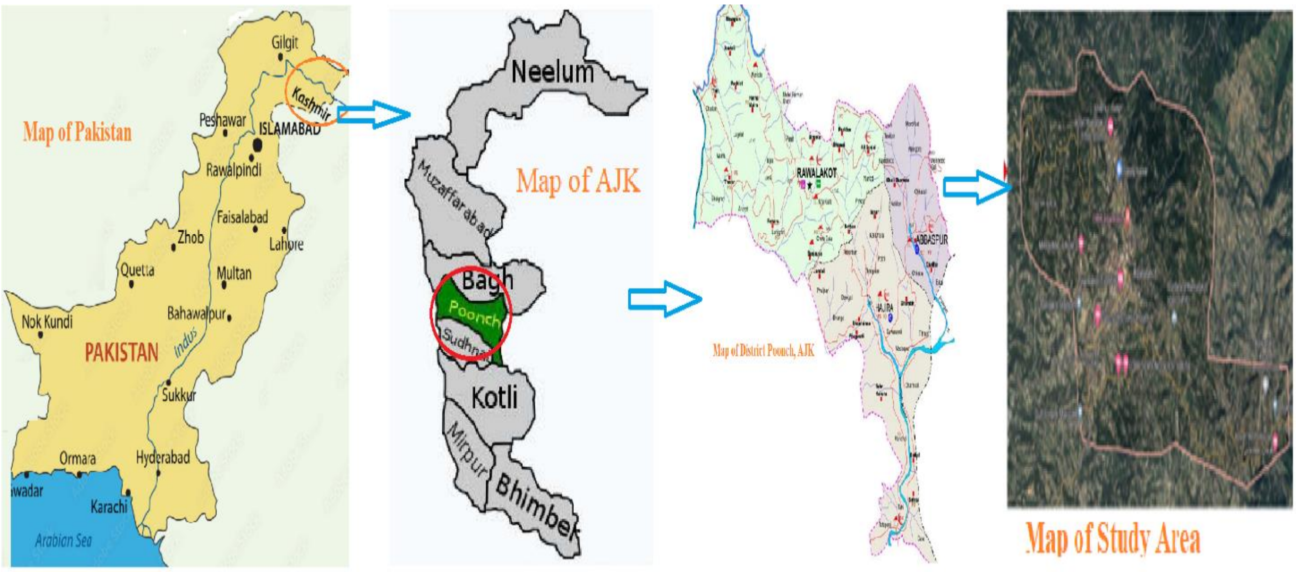

Fig. 1. Map of the study area surveyed for ethnobotanical and ethnomedicinal usws of *V. cruciatum* *https://geohack.toolforge.org/geohack.php?pagename=Rawalakot&params=33_51_12_N_73_45_05_E_region:PK_type:city*

The informants consisted of both genders of the inhabitants of the area and their total number was 90 (40 males and 50 female). Local name of *Viscum cruciatum*, mode of medicinal use, recipe and occurrence was asked from informants. Local recipes followed by inhabitants were recorded and cross checked with available literature for countersignature, particularly antidiabetic. Response of each interviewer was recorded for extracting concrete results and were supported by applying informant consensus factor (ICF) and fidelity level (FL) (Ishtiaq *et al*., 2021; Takht *et al*., 1996; Maqbool *et al*., 2019) which verified the information. This would be utilized for selection of plants for further ethnopharmacological analysis to discover novel drugs to combat against multidrug resistant (MDR) bacteria or other microbes which are causative of different diseases. The FL of this study was determined as prescribed below:

Fidelity level: FL is an index used which describes unanimous witness of informants on 'utilization of a specific plant for specific use or purpose'. FL is an indicator of popularity of use of specific plant as herbal drug being prevalently used to cure specific disease or used for any other purpose in the research area. FL was calculated using following equation (Maqbool *et al*., 2019; Ajaib *et al*., 2021).

$$FL = Np/N \times 100$$

where FL is represented in %; Np is number of respondents who claim "specific use of a particular plant" for cure of particular ailment or use in other purpose", and N describes the "total number of respondents" that use the plant for curing a particular disease or it any other use in the study area.

Informants' consensus factor: The Informants' consensus factor **(**ICF) is an EB index describing quantitative data about the "unanimous agreement of interviewees on use of TEMs for treating specific disease or group of diseases". It is determined by using following equation as per protocol of Maqbool *et al*., 2019 and Ishtiaq *et al*., 2021.

$$ICF = n_{ur} - n_t/n_{ur} - 1$$

where, n_{ur} represents "number of uses of a medicinal plant" for each category while n_t is the "total number of plant species used" to treat the particular group of infirmity or ailments. By following EPA model, on basis of ethnobotanical (FL and ICF indices) analysis, the folklore uses cited by indigenous communities of the area for *V. cruciatum* were evaluated and then selected for phytochemical analysis and activity (ethnopharmacological) based analysis.

Phytochemical analysis: The extraction of phytochemicals was completed using maceration process of Flanzy *et al*., (1987) and Maqbool *et al*., (2021) was followed. Fifty gram of leaf powder was dipped in 250mL MeOH and kept for 7 days and filtered, the liquid part was dried using rotary evaporator and dried residue was weighed to find the percentage yield (Ishtiaq *et al*., 2017).

The presence of different phytochemicals was testified by using different dedicated tests following Verma and Singh (2020). The Mayer, Wagner, Hager and Dragandrof test for Alkaloids, Ferric Chloride. Alkaline reagent and Lead acetate test for Flavonoids, Milon and Biuret test to find proteins, Foam and Bromine water test for Saponins, Ferric Chloride test for Tannins were performed following the procedures of Ingle *et al*., (2017) with little amendments (Ajaib *et al*., 2021).

Animal trials: To investigate versatility of *V. cruciatum* two host varieties *Olea ferruginea* Royle and *Quercus incana* on diabetic male rabbits (*Oryctolagus cuniculus* L.) were selected. Average weights of rabbits ranged within 1-1.5 Kg. after relocation of rabbits from animal house they were first kept within standard environment condition as per rules of Departmental Ethical Committee (DEC) of the University in line with international laws and rules. Permission for animal trials was sought from the Departmental Ethics Committee (DEC) of the University, which operates under the Advanced Studies and Research Board.

Afterwards test animals were brought to laboratory exactly two weeks before trial to adjust animal in laboratory environment it was compulsory to reduce stress levels in test animal. Throughout the time period test animal was fed with cabbage, grass, grains and adequate water.

For the preparation of diabetic model animals as trial treatments, procedure of Touseef *et al*., (2012) and Waheeda *et al*., (2016) was used for induction of diabetes in test animal and its treatment with *V. cruciatum*. Moreover, some changes were made as per requirements of experimental work. About 300 mg/dl of blood sugar level was considered as critical set point for indication of disease (Diabetes mellitus).

To achieve respective sugar level and diabetes confirmation, model animals were given various optimized doses of Alloxan monohydrate and 90 mg/kg dose was found to be effective unit to make our test animals diseased following Vieira *et al*., (2020). Further dilution of Alloxan with saline solution was primed and immediately injected in test animals. Alloxan shot was injected in marginal ear vein of test animal; before the injection of Alloxan ear was numbed with lignocaine, Wintogino balm and xylene this reduced pain sensation in test animals and xylene made veins prominent to give ease in injecting Alloxan. Further experimentation was done after confirmation of blood glucose level of test animals @ 300 mg/dL (Savych and Polonets, 2021; Mesfin *et al*., 2009; Waheeda *et al*., 2016).

Treatment groups of animals: Three major test groups were designed and named as Normal control group (NCG), Disease control group (DCG) and Treatment control group (TCG), TCG were further subdivided into different subgroups following Waheeda *et al*., (2016) which were designated names as below:

Group-I: which comprised of three rabbits with normal/control sugar level without any treatment that is non-diabetic.
Group-II: DCG in which diabetes has induced by alloxan dose but they remained untreated.
Group-III: TCG in which diabetes was induced by alloxan dose and they were treated with a commercially available medicine named 'glibenclamide' with the ratio of 0.2 mg/kg body weight.

Eight groups of TCG ($T_{group-1}$ to $T_{group-8}$) were subjected to different experimental trials were made depending on dose, host and plant parts variation and its details are enlisted below:

$T_{group-1}$: comprised of three rabbits which were treated with 500 mg of *V. cruciatum* (Host *Quercus*) MeOH extract of Leaf orally per day.
$T_{group-2}$: comprised of three rabbits which were treated with 1000 mg of *V. cruciatum* (Host *Quercus*) MeOH extract of leaf orally per day.
$T_{group-3}$: This comprised of three rabbits which were treated with 500 mg of *V. cruciatum* (Host *Quercus*) MeOH extract of bark orally per day.
$T_{group-4}$: This comprised of three rabbits which were treated with 1000 mg of *V. cruciatum* (Host *Quercus*) MeOH extract of bark orally per day.
$T_{group-5}$: This comprised of three rabbits which were treated with 500 mg of *V. cruciatum* (Host *Olea*) MeOH extract of leaf orally per day.
$T_{group-6}$: This comprised of three rabbits which were treated with 1000 mg of *V. cruciatum* (Host *Olea*) MeOH extract of leaf orally per day.
$T_{group-7}$: This comprised of three rabbits which were treated with 500 mg of *V. cruciatum* (Host *Olea*) MeOH extract of bark orally per day.
$T_{group-8}$: This comprised of three rabbits which were treated with 1000 mg of *V. cruciatum* (Host *Olea*) MeOH extract of bark orally per day.

Diagnostic Measurements

Blood glucose level: A glucometer (ACCU-CHEK-active model glucometer (Roche Co.) was used to measure blood sugar level of test animals on every third day during experimental trial following protocol of Waheeda *et al*., (2016). As Alloxan monohydrate was injected in ear veins so sugar was also tested by taking blood sample from ear.

Serum insulin level: To check serum insulin level approximately 3 cc. blood was taken from the thigh vein of rabbits on 7^{th} and 15^{th} day of experiment. Its serum was isolated by using centrifuge machine at 1000 rpm as per our established procedure in Lab (Waheeda *et al*., 2016). Serum was sent to Armed Forces Institute of Pathology (AFIP) Rawalpindi for further physiological analysis.

Body weight: On every third day of experiment body weight of rabbits was regularly checked by using digital weighing machine as per protocol of Touseef *et al*., (2012) and Waheeda *et al*., (2016).

Data analysis

To check the reliability of Ethnobotanical work, data were compiled in matrix form and analyzed using different statistical tools i.e., fidelity Level (FL), informant consensus factor (ICF) (Hanif *et al*., 2013; Maqbool *et al*., 2019; Ishtiaq *et al*., 2021). To analyze results of antidiabetic activity two-way ANOVA was performed using software "Graph pad prism 6". Its probability level was set at $p<0.05$ or less was considered as significant.

Results and Discussion

Plants have been part and parcel of human life and many wild plants have been used as source of medicines for curing different ailments. The correlation between man and plants is recognized as 'ethnobotany'. It describes all uses of plants in human life and their mutual impact in the environment (Gilani *et al*., 2009; Ishtiaq *et al*., 2021). Ethnobotany is considered to be the most pivotal method which helps to find ethnobotanical knowledge about wild plants, preferably ethnomedicinal usage. The traditional ethnomedicines (TEMs) potential and its authenticity are verified by using quantitative indices like FL and ICF (Saenz *et al*., 1997; Maqbool *et al*., 2019; Ishtiaq *et al*., 2021). Ethnobotanical research and traditional botanical knowledge is the demand of present world because people are turning towards herbal treatment due to its least side effects and less cost (Ishtiaq *et al*., 2021).

The plants named as 'misteletoes' are generally known as 'woody hemiparasites' which are present on plants of many families. Mostly this name is used for members of family Loranthaceae (Faulks, 1958; Bussing, 2000; Kienle *et al*., 2011). In ancient Europe folklore and religion, misteletoes got very prominent place before the start of Christianity. It was thought that these plants carry some magical powers and people could treat evils and superstitious powers with the help of these plants. These plants were also used for decoration purposes on New Year celebrations and on Chrismas (Parker *et al*., 1993). Mistletoe, belonging to the genus *Viscum* in the Loranthaceae family, holds significant cultural and medicinal importance across various societies. It has been associated with numerous traditions, particularly during winter holidays like Christmas and New Year's, where it is hung as a decoration, and kissing under it is believed to symbolize love and fertility (Dobrecky *et al*., 2022). In indigenous cultures, mistletoe is considered sacred, used in rituals, prayers, and ceremonies to invoke protection and blessings. Medicinally, mistletoe has been used for centuries in traditional practices, with extracts and infusions employed to treat ailments like hypertension, epilepsy and arthritis. In certain regions, mistletoe berries are edible, providing a traditional food source for some indigenous communities (Briggs, 2021). However, its toxicity requires caution in its use. Mistletoe's role in ecosystems is complex; while it can weaken host plants, it also provides habitat and food for various wildlife. Preserving mistletoe species and their habitats is crucial to maintaining their cultural, ecological, and medicinal significance (Briggs, 2021).

Traditional ethnomedicinal (TEM) survey: Data of TEMs of selected plant '*Viscum cruciatum'* was collected by interviewing 90 respondents of different age groups comprising of both genders. Plants were collected from different areas of Rawalakot, District Poonch of Azad Kashmir. Data was collected via open-ended and close-ended questionnaire method from indigenous people, practitioners (Hakims) and farmers. Through TEM survey it was found that '*Viscum cruciatum'* was potentially recognized as medicinal plants in the study are and it was prevalently used for curing different diseases like diabetes, hepatitis, skin diseases, cancer, veterinary medicine, mosquito repellent, liver and spleen infirmities, renal disorders and as antiplatelet agent to control hemophilia (Table 1). The collected TEMs data was cross-referenced with available literature to verify the authenticity of information. The TEM information provides clues for selection of plants for further qualitative and quantitative phytochemical analysis to explore its medicinal worth (Table 1). The plants have been used in rural cultural communities of Rawalakot, District Poonch of AJK for curing cancer, diabetes, tumors, muscular spasm and cancer (Table 1). This plant has also been used in many TEMs around the world for treating different diseases (Bussing, 2000; Lev *et al*., 2011; Szurpnicka *et al*., 2020).

Table 1. Traditional ethnomedicinal (TEM) uses of *V. cruciatum* from Rawalakot, District Poonch of Azad Jammu and Kashmir, Pakistan.

S. No.	Disease cured	Part used	Recipe	Phytochemical agent involved	Reference
1.	Diabetes	Leaf	Leaf extract or sometimes crushed powder is taken with milk twice a day.	Insulinotropic activity	(Gilani *et al*., 2009; Gupta, 2012; Turkkan *et al*., 2016)
2.	Mosquito repellent	Leaf	Leaf of plant is crushed and extract mixed with mustard oil is applied on hands and feet as mosquito repellent	Flavonoids	(Wesam *et al*., 2016)
3.	Platelet activity disorders	Plant bark	Bark decoction	Cholinergic and calcium channel	(Ahangarpour *et al*., 2014)
4.	Septic or infection/ inflammation	Leaf	Extract is used with honey for one week	Lectin, viscotoxin, phenols and non-polar compounds	(Ahangarpour *et al*., 2014)
5.	Cancer, tumors	Leaves	Crude extract	Lectins	(Ahangarpour *et al*., 2014)
6.	Spasmodic pain in muscles	Leaf and Bark	Extract or juice of plant acts as antispasmodic with little honey and other medicinal herbal extracts.	Saponins, flavonoids, alkaloids	(Wesam *et al*., 2016)
7.	Liver and spleen enlargement/ inflammation infirmities	Leaf and flower	Crude extract of leaf and flower is effect tonic when taken with honey	Coumarins, flavonoids, saponins, sterols, tannins, and terpenes	(Wesam *et al*., 2016)

Table 2. Fidelity level of wild plants of genus *Viscum* from Rawalakot, District Poonch of Azad Jammu and Kashmir, Pakistan used to cure diabetic medicine.

Plant	Host	Np	N	F.L% = NP/N×100
Viscum cruciatum	*Quercus incana*	48	70	68.57
	Olea ferruginea	51	70	72.86
	Acacia modesta	43	66	65.15
Viscum album	*Bombax cieba*	45	69	65.22
	Acacia nilotica	39	61	63.93
	Carissa opaca	30	48	62.50

According to respondents' data analysis, it is concluded that plant has prevalently been used as mosquito repellent and TEM medicine for diabetes cure (Table 1). Most commonly leaf and bark of plant are used for various medicinal purposes in different ethnic cultures of various areas of AJK. An EB quantitative tool FL was calculated for all wild plants of study area while here data two species of *Viscum* occurring on different hosts were presented while remaining data of other plants will be presented elsewhere in other research article (Table 2). An ethnobotanical index, Informant Consensus Factor (ICF) was calculated for each category of diseases to identify the agreement of informants on the reported cures for the infirmities (Table 3). ICF results predicted that the highest value (ICF=13.8) was recorded, followed by ICF value of 11.8 for mosquito repellent and Liver and spleen problems with ICF: 10.6 while the lowest value (ICF=1.70) was found for anxiety (Table 3). Based on highest value of FL, it was concluded that *Viscum cruciatum* occurring on host *Quercus incana* and *Olea ferruginea* was prevalently used in traditional ethnomedicines (TEMs) in the study area of AJK (Tables 2). ICF data analysis proved that the selected plant has great significance in herbal drugs based on hosts variations (Table 3); hence these two specimens were selected for further phytochemical study to authenticate the TEMs knowledge and neo drug discovery. The leaf, bark and flower parts were selected for further phytochemical analysis.

Phytochemical profiling: For phytochemical analysis, leaf, bark and flower parts were extracted in MeOH using maceration process. About 50 g of dried powder of *V. cruciatum* was dipped in 300 mL of 70% methanol, which was dried using rotary evaporator with 35.7 mg yield of green slightly sticky plant extract. This extract was further stored at -4ºC temperature for further experimental analysis using various phytochemical tests. Physical characteristics of leaf extract of *V. cruciatum* are listed in (Table 4). The leaf produced highest content of crude dried extract with yield of 9.10 % and the least yield (5.90 %) was recovered for flower powder. The obtained extract was of greenish in color with sticky in texture. Similar findings has been cited in previous researchers where it was proved that leaf mostly produced highest phytochemical extraction yield (Maqbool *et al*., 2017; Ishtiaq *et al*., 2007; Maqbool *et al*., 2021).

In qualitative analysis, MeOH extract of leaf kick marked the presences of strong phytochemical profile containing alkaloids, tannins, flavonoids, saponins and phenols. Beside these compounds terpenoids, cardiac glycosides and steroids were also found in plant extract (Table 5). The presence of these bioactive compounds in the plant parts of *Viscum cruciatum* proves that TEMs cited for this plant in different cultures have a scientific basis for curing of different infirmities. These findings are congruent with works of Maqbool *et al*., 2021; Ishtiaq *et al*., 2021).

Among phytochemistry results it was found that flavonoid, saponins, alkaloids, tanins, terpenoids and cardiacglycosides. The high concentration of different chemicals was detected in leaf followed by bark and flower (Table 5). As flower depicted very minute amount of phytoconstituents, hence it was not further used in other analytical tests. Similar results had been reported in the earlier works where it was stated that leaf had more conc. of flavonoids and other different chemicals (Ishtiaq *et al*., 2007; Maqbool *et al*., 2017; Pietrzak *et al*., 2017; Szurpnicka *et al*., 2020).

The flavonoids and terpenoids contribute to mistletoe's phytochemical profile, exhibiting antioxidant, anti-inflammatory, and antitumor activities. Polysaccharides found in mistletoes have been associated with immune-stimulating effects (Holandino *et al*., 2020).

Table 3. Informant consensus factor (ICF) of Villagers and Hakims (herbalists) of Rawalakot, District Poonch of Azad Jammu and Kashmir, Pakistan.

S. No	Disease category	Species (n_t)	All species (%)	Use citation (n_{ur})	Use citation (%)	ICF= n_{ur}-n_t/ n_{ur}-1
1.	Mosquito repellent	4	80	13	65	11.8
2.	Cancer	4	80	11	55	9.6
3.	Anxiety	1	20	3	15	1.7
4.	Hypertension	2	40	7	35	5.7
5.	Palpitation	2	40	7	35	5.7
6.	Diabetes	3	60	15	75	13.8
7.	Liver and spleen problems	2	40	12	60	10.6

Table 4. Percentage yield and physical characteristics of crude MeOH extract of different parts of *Viscum cruciatum* from Rawalakot, Azad Jammu and Kashmir, Pakistan.

Plant name	Parts used	Dry weight (g)	Weight of extract (mg)	Percentage yield (%)	Characteristics of extract	
					Color	Consistency
	Leaf	50 g	4.55 mg	9.10 %	Green	Slightly sticky
V. cruciatum	Bark	50 g	3.57 mg	7.14 %	Dark Green	Sticky
	Flower	50 g	2.95 mg	5.90 %	Green	Moderate sticky

Table 5. Phytochemical analysis of MeOH extract of *V. cruciatum* (Hosts *Quercus incana* and *Olea ferruginea*) from Rawalakot, District Poonch of AJK, Pakistan.

S. No	Phytochemicals	Tests performed	***Plant sample used V. cruciatum* Host *Quercus incana***			***Plant sample used V. cruciatum* Host *Olea ferruginea***		
			Leaf	**Bark**	**Flower**	**Leaf**	**Bark**	**Flower**
1.	Flavonoids	Ferric chloride test	+	++	+	++++	++	+
		Alkaline reagent test	+	++	+	++	++	+
		Lead acetate test	++	++	+	+	++	+
2.	Alkaloids	Mayer test	+++	+	+	+++	+	+
		Wagner test	++	+	+	++	+	+
		Hager test	++	+	+	++	+	+
		Dragandroff test	++	+	+	+	+	+
3.	Saponins	Foam test	+++	++	+	++++++	++	+
		Bromine water test	++	++	+		++	+
4.	Proteins	Millon test	+	++	+	+++	+	+
		Biuret test	+	++	+	+	++	+
5.	Tannins	Ferric chloride test	+	++	+	++	+++	+
6.	Cardioglycoside	Bromine water test	+++	++	+	+++	+	+
		Keller test	+	-	-	+	++	-
7.	Triterpenoids	Salkowaski test	+++	+	+	+	++	+
		Leibermann test	+	+	-	++	+	-
8.	Carbohydrates	Barfoed test	+++	++	+	++	++	+
		Benedict test	+	+	+	+	++	+

+++++ = excellent; ++++ = very good; +++ = good; ++ = average, + = poor, - = absent

+++++ = excellent; ++++ = very good; +++ = good; ++ = average, + = poor, - = absent

Table 6. Effect of different concentrations dose of *V. cruciatum* found on hosts *Quercus incana* and *O. ferruginea* on blood glucose level mg/dL of *Oryctolagus cuniculus* collected from Rawalakot, District Poonch of AJK Pakistan.

Days	N.C	D.C	TC	***V. cruciatum* from host *Quercus incana***				***V. cruciatum* from host *Olea ferruginea***			
				Leaf		**Bark**		**Leaf**		**Bark**	
				$T_{1\text{-group}}$ 500 mg	$T_{2\text{-group}}$ 1000 mg	$T_{3\text{-group}}$ 500 mg	$T_{4\text{-group}}$ 1000 mg	$T_{5\text{-group}}$ 500 mg	$T_{6\text{-group}}$ 1000 mg	$T_{7\text{-group}}$ 500 mg	$T_{8\text{-group}}$ 1000 mg
D0	124±0.9	524±2.1	443±6.9	464±4.4	440±3.0	448±5.5	482±2.8	482±2.0	441±9.1	440±8.3	455±3.3
D3	123±1.0	533±1.2	388±7.5	426±4.7	395±3.5	423±12.6	473±1.8	431±4.7	374±8.1	391±6.6	417±6.7
D6	123±0.6	539±1.0	316±11.5	365±4.0	335±6.3	379±2.9	461±4.5	341±8.8	332±8.2	353±6.7	372±7.5
D9	122±1.3	544±1.2	221±15.2	326±9.0	274±3.5	367±10.7	408±6.8	244±7.6	254±6.8	312±6.1	315±6.4
D12	123±1.3	547±0.3	160±4.8	224±6.4	225±5.8	335±8.4	343±13.1	192±8.6	187±7.2	251±1.5	252±8.2
D15	122±0.7	553±1.5	127±3.8	156±7.8	148±3.2	303±8.1	287±6.1	149±1.5	144±1.2	177±5.0	170±9.3

Anti-hyperglycemic analysis

Blood glucose level: Antidiabetic efficiency of *V. cruciatum* was evaluated using methanolic extract of leaf and bark collected from Hosts *Quercus incana* and *Olea ferruginea* on experimental animals (rabbits). Various concentrations (500mg and 1000mg) of dose were given to test animal to notify the appropriate dose concentration effectiveness against diabetics. Blood sugar level of test animal was monitored on every third days using glucometer.

For 20 consecutive days' blood sugar level of test animal was checked which depicted that increasing concentration of dose (extract) caused gradual decrease in blood sugar level. Rabbits treated with 1000 mg of *V. cruciatum* leaf extract from host *Olea ferruginea* showed maximum decrease in blood glucose level 144±1.2 ($T_{6\text{-group}}$) while minimum decrease was observed in group of animals treated with 500mg of bark extract of *V. cruciatum* from host *Quercus incana* 303±8.1 ($T_{3\text{-group}}$). Comparative analysis depicted that leaf extracts showed better results as compared to bark extracts and sample of *Olea ferruginea* showed better results than tghan *Quercus incana* host.

In-vivo studies of *V. cruciatum* revealed that alkaloids present in plant facilitates the hypoglycemic activity. As a hypoglycemic, it performs three primary actions: (i) it decreases hepatic glucose production, (ii) decreases intestinal absorption of glucose and (iii) increases efficiency of β-cell in insulin production (Ko *et al*., 2016). Conclusive result showed that 1000mg dose of leaves of *V. cruciatum* (on host *Olea ferruginea*) showed maximum decrease which was approximately equal to the test animals treated with allopathic medicine Glibenclamide tablets as shown in (Table 6).

Our results showed remarkable decrease in the blood glucose level in Alloxan induced diabetic rabbits was observed on day 15 of treatment. Treatment control group showed 71.4% decrease in blood glucose level when treated with leaves extract of *V. cruciatum* (Host *Quercus incana*) while treatment groups ($T_{1\text{-}4\text{-groups}}$) showed 56.5%, 68.66%, 68.75% and 69.7% decreases, respectively. Whereas 500mg methanolic extract According to these results *V. cruciatum* is found more effective in treating diabetics in test animal whereas further 1000mg leaf extract of *V. cruciatum* showed more significant results and close to the value of Blood glucose level of rabbits treated with commercial allopathic medicine, used as standard 'glibenclamide'. These results are also supported by the work of Michel *et al*., (2016). They also demonstrated that plants belonging to genus *Viscum* had very good hypoglycemic potential (Bussing 2000; Lev *et al*., 2011; Nna *et al*., 2013). In T 6-group, the best reduction in blood glucose level was reached upto 144±1.2, which proves that at day-15, with dose of 1000 mg/kg was the best optimized dose for controlling diabetes. These results are in agreement with previous works of Szurpnicka *et al*., (2020). This proves that plant has good potential for cure of hyperglycemic effects and it can be harvested for novel drug discovery through analytical techniques.

From the results of above-mentioned animal studies, it is indicated that V*iscum cruciatum* extracts may enhance insulin sensitivity, which can lead to better uptake of glucose by cells, ultimately resulting in reduced blood sugar levels (Ruiz *et al*., 2019). Additionally, the plant contains antioxidants, which play a crucial role in combating oxidative stress. Oxidative stress is linked to the development of diabetes and its complications, and the presence of antioxidants in *V. cruciatum* may help in reducing cellular stress associated with the condition (Nicoletti, 2023). Moreover, *Viscum cruciatum* has been studied for its potential to improve lipid profiles. Dyslipidemia, characterized by abnormal lipid levels in the blood, is often associated with diabetes. Some research has shown that the plant may lower total cholesterol, triglycerides, and LDL cholesterol levels, which could be beneficial for individuals with diabetes. Another area of interest is the plant's anti-inflammatory properties (Briggs, 2021). Chronic inflammation is a contributing factor in insulin resistance and the progression of diabetes. Components of *Viscum cruciatum* have demonstrated anti-inflammatory effects, which may play a role in managing diabetes and its complications (Ruiz *et al*., 2019).

Measurement of weight of rabbits: Body weight has great influence in insulin inhibition and its metabolism disorders. Obesity increases the risk of diabetes occurrence in human being. Along with hypoglycemic activity bioactive compound of *V. cruciatum* also reduces body weight, as shown in results (Table 7). Body weight of test animals was checked after every third day by Digital Weighing Machine to record effects of MeOH leaf and bark extracts of *V. cruciatum*. For 20 consecutive days the weight of test animal was checked and it was monitored that on 15th day, weight of test animal treated with 1000 mg leaf extract of *V. cruciatum* (Host *O. ferruginea*) showed maximum decrease in weight which was 0.884±0.02 whereas, minimum decrease (1.45±0.031) was observed in 500mg bark extract of plant from host *Q. incana* and these herbal extracts' results were very promising and near to treatment control group reading 0.98±0.014 (Table 7). Plants obtained from host *O. ferruginea* were found more effective compared to plants obtained from host *Q. incana*. These findings proved that when impact of diabetes was reduced due to active rise of insulin in body by natural mode then body weight was increased as glucose was stored in the form glycogen and similar findings were also repoted in previous works where herbal therapeutics had proven potential candidate for diabetes disease control (Akueshi *et al*., 2002; Bouche *et al*., 2004; Waheeda *et al*., 2016).

Measurement of serum insulin level: Another parameter 'Serum insulin level' of test animals was examined to evaluate the salvage of animals from diabetes at 7th and 15th days of experiment. Different Methanolic extract concentrations of leaf of *V. cruciatum* parasitising different hosts were analyzed. The results exhibited that serum insulin measured at 15th day of test animal canned with 1000mg leaf extract of *V. cruciatum* (Host *O. ferruginea*) showed increase in serum insulin level up to 5.79±0.11 ($T_{6\text{-group}}$) and this was the maximum increase and minimum increase was observed in rabbits treated with 500mg bark extract of plant from host *Quercus*. Results showed that significant increase in insulin level was observed in rabbits treated with 100 mg leaf extract dose and it has revealed that the plants from *O. ferruginea* were more effective compared to *Q. incana* as shown in (Table 8). It is stated in previous research works that blood glucose decrease or increase is associated with insulin level in blood serum as well as it is linked with health and number beta cells of pancreas (Ullah *et al*., 2022). In experimental rabbits in which diabetes was induced by alloxan dose led towards loss of Beta cells and sugar control in serum and blood was maintained due to dose of leaf and bark extracts of *Viscum cruciatum*. The research work outcome of the present work was that herbal treatment of *Viscum cruciatum* reduced blood glucose in serum and these findings were in accordance of previous researchers (Wadood *et al*., 2007; Waheeda *et al*., 2016).

The current findings of research proved that plants of *Viscum cruciatum* growing on both the hosts *Q incana* and *O ferruginea* have very good potential for controlling diabetes. The results outcomes are evidenced from ethnomedicinal studies which are authticated by indices of FL and ICF which on phytochemical profiling proved presence of many valuable phytoconstituents who are candidate agents for actual curing of the hyperglycemia. Thus, our Ethnobotanical (E) survey, Phytochemical (P) analysis and ethnopharmacology activity (A) based experimental technique called "EPA" were proved to be a very useful approach for selection, extraction of phytochemicals and activity/experiment for diabetes control. This has already been used in previous studies by Ishtiaq *et al*., (2017); Bussing (2000); Szurpnicka *et al*., (2020); Maqbool *et al*., (2021).

Furthermore, there are indications that *V. cruciatum* extracts may protect pancreatic beta cells. These cells are responsible for producing insulin, and their damage or dysfunction is associated with diabetes. The plant's potential to protect these cells could help maintain insulin production and regulation (Park *et al*., 2019). In addition to the above-mentioned effects, *V. cruciatum* has been studied for its ability to inhibit the formation of advanced glycation end products (AGEs). AGEs are formed when proteins and sugars combine non-enzymatically and contribute to diabetic complications. Inhibiting their formation could potentially reduce the risk of diabetic complications (Szurpnicka *et al*., 2020).

Table 7. Effects of various extractions of *V. cruciatum* from hosts *Quercus* and *Olea ferruginea* on weight of test animal *O. cuniculus* (gm).

Days	N.C	D.C	TC	*V. cruciatum* from host *Quercus incana*				*V. cruciatum* from host *Olea ferruginea*			
				Leaf		Bark		Leaf		Bark	
				$T_{1\text{-group}}$ 500 mg	$T_{2\text{-group}}$ 1000 mg	$T_{3\text{-group}}$ 500 mg	$T_{4\text{-group}}$ 1000 mg	$T_{5\text{-group}}$ 500 mg	$T_{6\text{-group}}$ 1000 mg	$T_{7\text{-group}}$ 500 mg	$T_{8\text{-group}}$ 1000 mg
D0	1.31±0.20	1.48±0.03	1.22±0.23	1.47±0.08	1.35±0.31	1.58±0.20	1.53±0.10	1.45±0.07	1.38±0.03	1.47±0.02	1.45±0.06
D3	1.32±0.15	1.47±0.03	1.20±0.22	1.46±0.03	1.33±0.23	1.56±0.28	1.52±0.11	1.39±0.04	1.31±0.05	1.39±0.31	1.43±0.04
D6	1.32±0.02	1.47±0.25	1.17±0.02	1.43±0.05	1.31±0.09	1.53±0.15	1.50±0.09	1.31±0.01	1.25±0.03	1.34±0.09	1.37±0.08
D9	1.32±0.22	1.46±0.03	1.15±0.03	1.39±0.04	1.27±0.26	1.50±0.19	1.49±0.10	1.28±0.03	1.17±0.03	1.30±0.01	1.31±0.01
D12	1.33±0.10	1.39±0.06	1.13±0.03	1.32±0.05	1.25±0.28	1.48±0.20	1.46±0.15	1.21±0.02	1.12±0.05	1.25±0.07	1.28±0.02
D15	1.34±0.15	1.35±0.03	0.98±0.14	1.28±0.05	1.22±0.18	1.45±0.31	1.43±0.09	1.11±0.03	1.03±0.37	1.16±0.03	1.14±0.03

Table 8. Insulin concentration in serum of *O. cuniculus* treated with different methanolic extract concentrations of *V. cruciatum* from hosts *Q. incana* and *O. ferruginea* (pmol/L).

Days	N.C	D.C	TC	*V. cruciatum* from host *Quercus incana*				*V. cruciatum* from host *Olea ferruginea*			
				Leaf		Bark		Leaf		Bark	
				$T_{1\text{-group}}$ 500 mg	$T_{2\text{-group}}$ 1000 mg	$T_{3\text{-group}}$ 500 mg	$T_{4\text{-group}}$ 1000 mg	$T_{5\text{-group}}$ 500 mg	$T_{6\text{-group}}$ 1000 mg	$T_{7\text{-group}}$ 500 mg	$T_{8\text{-group}}$ 1000 mg
D7	10.20±0.26	0.00±0.00	4.43±0.17	1.19±0.08	2.62±0.3	1.3±0.06	1.45±0.04	3.5±0.17	3.26±0.50	3.0±0.31	3.37±0.28
D15	10.20±0.21	0.01±0.003	7.63±0.30	2.03±0.04	3.33±0.3	1.63±0.03	1.89±0.01	5.6±0.15	5.79±0.11	4.17±0.24	4.83±0.18

Conclusion

The research on the medicinal potential of *V. cruciatum* occurring on different hosts in Rawalakot, District Poonch of AJK revealed its multifarious usage in green medicines. The findings indicate that these plants can serve as traditional ethnomedicines for treating diabetes and other prevalent infirmities in the study area. The universal use of *V. cruciatum* in traditional ethnomedicines for diabetes control suggests a promising direction for novel drug development targeting hyperglycemia and other related health issues. The plant's high medicinal worth is attributed to the presence of various phytochemical compounds, which contribute to regulating blood sugar levels through increase of insulin production, enhanced glucose absorption by muscles and fat tissues, and reduction of glucose absorption in the intestine, along with lower glucose production by the liver. The recent studies, along with existing literature, provide strong evidence of *V. cruciatum*'s anti-diabetic abilities.

Future perspectives: Further research is warranted to explore the potential of *V. cruciatum* using different solvents and to investigate its various samples occurring on diverse host plants. This expanded ethnopharmacological research could pave the way for neo drug discovery and the development of innovative medicines. Additionally, comparative studies on the efficacy of *V. cruciatum* occurring on different host species would be valuable in understanding the variations in its anti-diabetic properties. In view of very effective results obtained for *V. cruciatum* Host *O. ferruginea* against Type 2 diabetes, it is highly recommended that diabetic and pre-diabetic individuals consider this herbal therapy as an alternative to insulin injections and other allopathic treatments. Embracing such natural remedies may offer promising health benefits and potentially improve the management of diabetes and related health conditions in the study area and beyond.

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