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Phytochemical analysis and pharmacological applications of *Berberis lycium*

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Abstract
All the bioactive compounds isolated from the roots of *Berberisl ycium* showed the presence of alkaloids, terpenoids, tannins, saponins, reducing sugar as major secondary metabolites. Berberis lycium contain variety of phytochemicals like steroids, flavonoids, alkaloids, tannins, anthoquinonine, Terpenoids, proteins, and carbohydrates. Some other phytochemicals isolated from same plant were Barberine, Berbericine hydrochloride, Berberine chloride, Berberine chloroform, chenabine, diphenolic, palmatine, Jhelumine, Karakoramine, palmitive chloroform along with oxyberberine, punjabine, seco-bisbenzy lisoquinoline, sindamine, umbellatine etc

Keywords: Phytochemicals, pharmacological applications, *Berberis lycium* etc.

Introduction
The plant was described under the name of Ambaribis by Al-Biruni. He also mentioned its Persian name as Zirkash. Its common name is Kashmal. However, it is called Ishkeen and Sumbal in the area of its collection. (Said *et al*, 1996) [1]. Berberis lycium was described in 1837 by John Forbes Royle. *Berberis lycium* commonly known as Barberry belongs to the genus Berberis of family Berberidacae. It sustains its leaves in all seasons. It produces flowers from May to June. Its flowers are bisexual and pollinated by insects. The plant is widely used for medicinal purposes. A common recipe is to boil sliced pieces of root and its bark in water. The water extract is strained and further boiled until a semi solid mass, called "Rasaut" is obtained. The extract of roots is used for the treatment of urinary tract infections, enlargement of spleen, gastric and duodenal ulcer and liver disorders. The product is mixed with butter and alum to be used as an external application for the eyelids in acute conjunctivitis. Similar ointment containing camphor is used against acne, pimples and other skin infections. The main biological activities are attributed to its principal constituent, berberine (an alkaloid). The compound is bitter in taste and imparts the Rasaut unpleasant taste. To make it palatable, Rasaut is sometime mixed with sugar and maize like meal. This cooked recipe is called "Halva". The "Halva" thus formed, is used regularly as a remedy by rheumatic patients for a longer period of time. The local inhabitants also use the dried mass of the root bark in powder form after mixing with molten animal fat as bandage for bone fractures. In Indochina, the fruist is given as a cure for renal disorder. The fruit juice is used for gums and teeth ailments. Decoction of fruit is used in typhoid and common cold. Stems of the plant are used for the stomach pain, diarrhea, jaundice and in the inhibition of melano hialdehyde (MDA). The bark of the plant shows wound healing activity. The plant leaves are used as tea substitute. The fruits are in the form of berries and are used as fresh or in dried form. The plant on the whole is used by the local inhabitants for the treatment of swollen and sore eyes, broken bones, internal injuries, ulcer, juindice and rheumatism. Previous investigations on this plant showed isolation of different types of compounds including alkaloids and steroids. Some biologically important isolated compounds are berberine, palmatine, Berbamine, Aromoline, Oxyacanithine, Umbellatine, ß-sitosterole, Punjabine, Balochistanamine, oxyberberine, berberinechloroform and palmatine chloroform. Berberine and its analogues represent a structural class of organic cations, isolated from numerous plants of Genera Berberis, Mahonia and Coptis. They have been shown to exert a broad spectrum of antimicrobial, anticarcinogenic and antimutagenic activity. In Pakistan Berberis lycium is also of great importance as a house hold remedy for the treatment of various

diseases. Antioxidants are substances which scavenge or eliminate free radicals from living bodies. The main purpose of the antioxidants is to slow down or eliminate the oxidation processes. The ultimate end of oxidation processes is to produce free radicals. These free radicals produced inside the living bodies are the main source of cancer cells. Antioxidants terminate oxidation processes by removing free radical intermediates by being oxidized themselves. The antioxidants are basically the reducing agents. It was thought that antioxidants are useful for better health but the large clinical trials did not detect any benefit. Afterwards it was suggested that the excessive use of antioxidants may be harmful. The antioxidants can be used industrially as well. Antioxidants are used as food preservatives, in cosmetics and preventing the degradation of rubber and gasoline. The chemical structure of all the antioxidants contains hydroxyl groups which actually take part in the oxidation reduction process. Antioxidants are classified as naturally occurring antioxidants and synthetic antioxidants. Naturally occurring antioxidants are some minerals, vitamins and phytochemicals. Minerals containing cupper, iron, zinc, maganese and selenium metals are useful antioxidants. The vitamins are vitamin B, vitamin C and vitamin E. Phytochemicals are mostly flavonoids. Synthetic antioxidants are mostly phenolic compounds that perform the function of scavenging and capturing free radicals. These include butylated hydroxyl anisole (BHA), butylated hydroxytoulene (BHT), propyl gallate and metal chelating agent (EDTA), tertiary butyl hydr2oquinone (TBHQ) and nordihydro guratic acid (NDGA). The chemical structure of all the antioxidants contains hydroxyl groups which were helpful for oxidation reduction processes. Keeping in view the therapeutic effect of *Berberis lycium* the roots of the plants were subjected for phytochemical studies. The isolated compounds after identification showed that they contain hydroxyl groups. Due to the presence of hydroxyl groups in the compounds including different extracts were subjected to antioxidant activities. The earlier research showed only the antioxidant studies of plant extracts. The antioxidant activities of the fruit of *Berberis lycium* was studied voltametrically. There were no reports of antioxidant activities of root extracts of this plant. The details of the structural effect on free radical scavenging was tried to study in this report. The main focus of the research was to find whether hydroxyl groups present on the isolated compounds were able to scavenge free radicals or not.

Berberis lycium

Occurrence: The plant is generally found in Himalayan regions of India, Pakistan and some part of Nepal.

Nomenclature: The plant is known as "Kimal" in the local area of extraction that is in Doda area of J&K, India. Apart from this the commonly used names of this plant are:

English: Indian Barberry
Hindi: Kashmal or Kasmal
Urdu: Ishkeen

Taxonomical classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnaliopsida
Family: Berberidaceae
Genus: Berberis
Species: lycium

Phytochemistry

Phytochemistry is the branch of chemistry that deals with the study of phytochemicals. It can be considered as abridging branch between botany and chemistry. This branch investigates the structures, function pharmacological activities of phytochemicals and their synthesis for further use. Phytochemistry thus revolves around the investigation of phytochemicals.

Phytochemistry deals with the methods of extraction, separation, purification and identification of different constituents present in the plant; thus it involves the exploration of old and new advanced techniques to understand the nature and functions of the active compounds present in the plant.

Phytochemical analysis begins with the extraction of phytochemicals or in common language we can say the digging out of the constituents present in the plants. To extract the constituents from the plant material a researcher has to make sure that the constituents are not destroyed or disintegrated while the extraction process and can be isolated easily from plant extract. There are various standard methods to carefully extract the constituents depending upon the nature of plant and the part of the plant utilized. Generally the plant part to be extracted is separated carefully from plant and dried for 72 hours in shady area in sunlight. After it is dried it is crushed into powdered form, further the powdered material is dissolved into appropriate solvents and thus a plant extract is formed which is labeled and taken to laboratory for further investigations. Alcohol is widely used as a solvent for the initial extraction processes. Polarity of the solute is kept in mind while choosing the solvents used for extraction of bio molecules. Polarity order of some of the commonly used solvents in order of increasing polarity is given below:

Hexane< Chloroform< Ethyl acetate< Acetone< Methanol< Water

Material and Methods

General laborartory and aseptic techniques as described in Dodds and Roberts (1984) were followed. Aseptic techniques were carried out in a Laminar Air Flow Bench (Klenzaids, India) equipped with a germicidal UV lamp.

Clean glassware of Corning or Borosil brands were used. They were washed in acidified dichromate, detergent and in running tap water. Glassware were rinsed in distilled water and dried before use. Pre-sterlised plastic were used in this study from Tarsons (India), Laxbro (India) and Falcon (USA) brands. Distilled water of reagent grade was used for the preparation of all the solutions and reagents.

Laboratory grade chemicals from Glaxo or SDS (India) were used for preparation of tissue culture media. Analytical grade chemicals were used for preparation of reagents and solutions. Salts were weighed using a monopan balance (Sartorious, Germany).

The medicinal shrub used in this study is *Berberis lycium*. The root samples of the plant were collected in good condition, without any mechanical, biological or microbiological damage from the Chiralla area of Doda district, Jammu and Kashmir, India in July and August months of 2019.

Materials and Methods

Roots of ***Berberis lycium*** were collected from district Doda, (Jammu and Kashmir) India. The plant was identified by Botanical section of University of Jammu. The roots were

thoroughly washed with water to remove dust and other contaminants. The clean roots were dried under shade at room temperature for two months. The roots were assumed completely dried, as there was no significant change in weight with the time. They were cut into small pieces and then pulverized into fine powder. 2kg of powdered material were extracted three to four times with 8 liters of Pet ether for fifteen days. After every extraction, plant material was removed from solvent by decantation. The extract was evaporated to obtain 0.21g of dry residue. Plant material, after ether extraction, was again dried and further extracted with methanol two to three times for a month with total 13 liters of the solvent. After that it was filtered. Methanol extract was evaporated similarly under reduced pressure to yield 350ml of dark brown syrup of methanol extract. Methanol extract was mixed with 750ml of 5%hydrochloric acid. Supernatant along with precipitates, formed on the acid addition, were kept in refrigerator overnight and then filtered. These precipitates after drying, weighed 10.2 g representing compound (berberine). The filtrate (1025ml) was adjusted to pH 8-10 by adding 30% ammonia. The precipitates formed under basic conditions were allowed to settle down in the form of salt and filtered. The filtrate was extracted with 10 liters of chloroform.

Chloroform extracts were evaporated on rotary evaporator to yield 10g of chloroform residue. The pH of aqueous layer was adjusted at 7 by the addition of HCl. This neutral aqueous layer was extracted with 6 liters of n-Butanol. n-Butanol extracts were evaporated on rotary evaporator to yield 12.1g of dry residue. The pet ether extract was evaporated under reduced pressure to yield 0.21g of dry extract. This dry extract was than subjected to fractionation by column chromatography. Elution was made with Pet ether / Ethyl acetate (6:4). It afforded one pure compound (ß-sitosterol) and other impure fractions were collected and again subjected to fractionation by passing through a column. Elution was made with Pet ether / ethyl acetate (8.5:1.5). Another pure compound was obtained which is (4, 4dimethylhexadeca-3-ol). 10 g of dry chloroform extract was dissolved in CHCl3, adsorbed on silica and was subjected to column chromatography over silica gel eluting with n-hexane/chloroform in increasing polarity to get Compound (butyl-3-hydroxypropyl phthalate) and (3(4'-(6-methyl butyl) phenyl) Propan-1-ol). The remaining impure fractions were again collected and subjected to column chromatography using chloroform/methanol in increasing polarities to get compound (3-(4'-(6-methyl butyl) phenyl) Propan-1-ol). Spectral Techniques Melting points were recorded using a digital Gallenkamp (SANYO) model MPD.BM 3.5 apparatus. IR spectra were recorded on AT-FT-IR Spectrophotometer (Omnic) using KBr pellets.1H- and 13C-NMR spectra were recorded on Bruker spectrometers (AM-300). Chemical shifts have been reported in parts per million (ppm) relative to SiMe4.The coupling constants are reported in Hz. Mass spectra was acquired (EI, 70eV) recorded on Mass Spectrometer Mat 312 system. The purity of isolated compounds was checked on pre-coated TLC plates and by their sharp melting points.

Antioxidant Activity of plants extracts and isolated compounds

Stable DPPH (2, 2 Diphenyl, 1-picrylhadrazylhydrate) radical has been used as a reactive compound to measure antioxidant activity When DPPH reacts with an antioxidant the reaction proceeds with change in color (from deep violet to light yellow), which can be measured at 518nm. The extracts and isolated compounds were tested in the DPPH free radical scavenging test by modified method outlined in literature. Solutions of four extracts (pet ether, Methanol, chloroform, and n-Butanol) and isolated fractions of Pet ether, chloroform and methanol were prepared in methanol and assayed for antioxidant activity. 20mg of dry extract and isolated fractions were mixed with 20ml of methanol in each case. The solution of DPPH (100µM) was also prepared in methanol. 2.5ml of DPPH solution was mixed with 0.5ml of extract solution. The mixture was kept in dark for one minute and then decrease in absorbance at 518nm was monitored after every one minute for fifteen minutes. Solution containing 2.5ml DPPH and 0.5ml methanol was used as a blank. The solution of Gallic acid (10µM) was used as a positive control. Radical scavenging activity was calculated by following formula.

Antioxidant Activity of *Berberis lycium* extracts the antioxidant activities of petroleum ether, methanol, chloroform and n-Butanol extracts were studied by means of DPPH free radical discoloration method. The Gallic acid was used as a standard for positive control and DPPH as radical scavenger. Antioxidant Activity of Isolated compounds. The antioxidant activities of berberine, β-sitosterol, 4,4 dimethyl hexadeca 3-ol, butyl -3-hydroxypropyl phthalate and 3-[4-(6-methyl butyl) phenyl] propan-1-ol were also subjected for antioxidant assay. The Gallic acid was used as a standard for positive control and DPPH as radical scavenger.

Observation and Result

The phytochemical analysis of *Berberis lycium* was conducted and the results observed are presented in tabular form;

Table 1: Observation

Phytochemical	Test Used	Observation	Inference
Alkaloid	Wagner's test	Brown flocculants precipitate observed	Present
Terpenoid	TLC	Colored spots of monoterpens	Present
Saponins	Froth formation method	Emulsion is formed	Present
Phenolic Acid	Chromatographic method	Minute color change	Present
Flavonoids	NaOH test	Yellow color appeared	Present
Tannins	Ferric chloride test	Blue black color observed	Present
Glycoside	Fehling's test	Red precipitates formed	Present
Reducing sugar	HCL test	Brown solution is formed	Present
Steroids	Chloroform test	Color changed from violet to green	Present
Amino Acids	Thin layer chromatography	Violet purple color observed	Present

The extract of *Berberis lycium* root showed the presence of the phytochemicals like alkaloids, glycosides, phenolics, saponins, tannins, flavonoids, terpenoids etc. Apart from these diheptanoids were present in very low negligible quantity.

Discussion

Berberine Mass (low resolution) spectrum of the compound afforded molecular ion peak at m/z 337.1 corresponding to molecular formula C20H18NO4 (Calculated=337.12). This compound was identified as berberine. It is an alkaloid. ß-sitosterol Mass (low resolution) spectrum of the compound afforded molecular ion peak at m/z 414.2 that confirmed the molecular formula C20H18NO4 (Calculated=414.2). This spectral data verified that data reported in literature of compound ß-sitosterol. 4,4-dimethylhexadeca-3-ol Mass (low resolution) spectrum of the compound showed a peak at 270 that confirmed the molecular formula C18H32NO (Calculated=270.2922). On the basis of above mentioned structural data compound was identified as 4,4-dimethylhexadeca-3-ol.

Conclusion

In the area of extraction of plant, *Berberis lycium* is used by locals for many medicinal purposes like, common cold, cough, eye complaints, chronic diarrhoea, jaundice etc. Apart from this some of them use it curing early stage diabetics. *Berberis lycium* is a versatile shrub with lots of medicinal properties. The leaves are used in treatment of jaundice, in addition to that Rhizome of Berberis species have antibacterial effects, oral treatment of it is used for various enteric infections especially bacterial dysentery. The different parts of the plant are known to prevent eye disorders, abdominal disorders, skin diseases etc. Plant is extensively used in local practices for the treatment of several human diseases like piles, menorrhagia, jaundice, wounds and broken bones.

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