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ORIENTAL JOURNAL OF CHEMISTRY
An International Open Free Access, Peer Reviewed Research Journal

www.orientjchem.org

ISSN: 0970-020 X
CODEN: OJCHEG
2018, Vol. 34, No.(2):
Pg. 1055-1062

Pharmacological Studies of Root, Fruit and Flower Extracts of *Berberis lycium*

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http://dx.doi.org/10.13005/ojc/340257

(Received: January 18, 2018; Accepted: March 02, 2018)

ABSTRACT

Herbal plants have many bioactive compounds in their different parts which are responsible for biological activities in the body like antimicrobial, antioxidant, anti-inflammatory properties etc. *Berberis lycium* is one of the most rare herbal plant found in the Garhwal region of Uttarakhand. In the present work different extracts of fruit, roots and flowers of *Berberis lycium* belonging to the family of berberidaceae were studied and compound were identified as antimicrobial and antioxidant activities were determined. The study revealed the presence of some biologically active components like, 2'-Phenylbenzanilide, Benzamide, N-(1,1'-Biphenyl)-4-yL-, Benzamide, N,N-Diphenyl- etc were detected in flower, fruit and root extract after GC-MS analysis. Methanolic extracts have shown good antimicrobial potential which can be used in medicines after further study in future.

Keywords: Antimicrobial activity, Extract, Berberidaceae, antioxidants.

INTRODUCTION

Plant kingdoms are the rich source of organic compounds, many of which have been used for medicinal purposes. In traditional medicine, there are many natural crude drugs that have the potential to treat many disease and disorders.[1-2]

The Himalaya and its region is rich in terms of herbal plants. Uttarakhand, which includes the major divisions of Kumaun and Garhwal, is a part of Indian Himalayan region and hence is endowed with rich biodiversity. As medicinal plants receive increased scientific and commercial attention, there is increasing pressure on the wild plant populations from which most of the medicinal plants are harvested. Overharvesting of these medicinal plants have led some of the species to become endangered[3-4].

Berberis lycium is an evergreen, erect shrub belonging to the family Berberidaceae. The genus Berberis comprises of as many as 500 species. Out of which 77 species are native to India [5]. *Berberis lycium* grows upto a height of 3m, with a thick woody shoot and covered with a thin brittle bark. *Berberis lycium* has bright yellow color flowers, which are hermaphrodite in nature; i.e. both male and female organs are present on the same flower. It is pollinated by insects. The fruits are bluish purple in color on ripening. The fruit is slightly acidic and juicy in nature. The plant possesses wide ecological amplitude and seeds can be grown in sandy, silty or loamy soils[6].

In this study fruit and flower extracts in addition to root extracts, were taken for detection of bioactive compound and pharmacological activity due to them.

MATERIAL AND METHODS

Collection of samples

The roots, fruits and flowers were collected from Garhwal region of Uttarakhand during summer season of May to July and preserved in the advanced chemistry lab of Uttaranchal University, Dehradun.

Extract preparation

The roots, fruits and flowers were collected separately and were washed thoroughly with running tap water and finally with distilled water. After shade drying for 10-15 days the roots were crushed into powdered form with the help of mortar and pestle. Then it was added to soxhlet assembly for extraction using water, methanol and n-hexane. It was heated for about 6 h at a temperature less than the boiling point of the solvent. The extract was further concentrated by rotary evaporator, residue was stored for further process where as in case of aqueous media same amount of sample was dissolved in water and boiled, filtered and saved for further process [7].

Collection of bacterial strains

Total 12 microbial cultures belonging to 8 bacterial species and 4 fungi were used in this study. The identified microorganisms were obtained from Microbiology laboratory, Uttaranchal University, Dehradun.

Antimicrobial activity and antioxidant activity

The root, fruit and flower extracts of *Berberis lycium* prepared above were again dissolved in similar methanol, n-hexane and water (100mg/ml) and sterilized. The antimicrobial activity test was carried out by disk diffusion by using 50ìl of suspension spread on potato dextrose agar (PDA), Mueller hinton agar (MHA) media respectively. The disks (6 mm) containing 10 µl of extracts (300 µg/ disk) with the concentration of 100mg/ml were impregnated in the inoculated agar. Negative control was prepared by using similar solvents of plant extracts. The inoculated plates were incubated at 37 °C for 24 h in the case of clinical bacteria strains, 72 h for fungi isolate. First, inhibitory activity was tested in different solvent extracts of root, flower and fruits. Solvent extracts which have shown positive antimicrobial activity were taken for detailed zone of inhibition by disc diffusion method. Antimicrobial activity was assessed by measuring inhibition zones in reference to test organisms and each process was repeated to get more precise results. Antioxidant activity of the selected plant extracts were measured by the Enzymatic Antioxidant and Non- Enzymatic Antioxidants methods. The enzymatic antioxidant properties were determined by superoxide dis-mutase (SOD), and Non Enzymatic Antioxidant property was determined in the form of vitaminC. i.e ascorbic acid and beta-carotene bleaching method [8-9].

Observations

The results observed are presented in the form of tables are given below. Antimicrobial effect have been measured in terms of zone of inhibition (mm), which is given in Table 1 and antifungal effect have been shown in Table 2.

Table 1: Zone of inhibition (diameter in mm) shown by extracts of *Berberis lycium* against selected bacterial strains

Microorganisms (Bacteria)	Root Aqueous	Root Methanol	Root n-hexane	Fruit Methanol	Fruit Aqueous	Fruit n-hexane	Flower Aqueous	Flower Methanol	Flower n-hexane
Escherichia coli (Gram Negative)	ND	ND	ND	ND	ND	ND	13	13	ND
Pseudomonas aeruginosa (Gram Negative)	14	13	11	14	16	16	17	20	18
Salmonella typhi (Gram Negative)	16	17	15	ND	ND	ND	ND	17	ND
Proteus mirabilis (Gram negative)	ND	ND	ND	12	12	13	13	17	14
Acinetobacter baumannii (Gram negative)	ND	16	ND	ND	13	ND	20	12	ND
Staphylococcus aureus (Gram positive)	12	ND	ND	ND	14	ND	14	19	ND
Enterococcus (Gram positive)	ND	ND	ND	ND	15	16	16	28	18
Klebsiella pneumoniae (Gram negative)	ND	14	13	13	13	12	12	15	14

ND-Not detected

Table 2: Zone of inhibition (diameter in mm) shown by extracts of *Berberis lycium* against selected fungal strains

Microorganisms (fungi)	Root Aqueous	Root Methanol	Root n-hexane	Fruit Methanol	Fruit Aqueous	Fruit n-hexane	Flower Aqueous	Flower Methanol	Flower n-hexane
A.cuboida	ND	ND	ND	ND	ND	ND	ND	16	ND
A.niger	ND	ND	ND	13	15	12	ND	14	ND
A.fumigatus 13	ND		12	10	ND	ND	ND	ND	14
Candida albicans	ND	11	8	ND	ND	ND	ND	ND	ND

Table 3: Enzymatic antioxidant acitivity (SOD)

Part used	Solvent extract	SOD (unit/mg of protein)
Roots	Aqueous	18.5
Roots	methanol	10.2
Roots	n-hexane	19.7
Fruit	Aqueous	20.1
Fruit	methanol	21.0
Fruit	n-hexane	18.9
Flower	Aqueous	16.0
Flower	methanol	15.8
Flower	n-hexane	15.2

Table 4: Antioxidant activities of extract with DPPH assay

Plant Part	Solvent extract	DPPH Inhibition %
Root	Aqueous	72
Root	Methanol	62
Root	n-hexane	78
Fruit	Aqueous	65
Fruit	Methanol	55
Fruit	n-hexane	70
Flower	Aqueous	56
Flower	Methanol	48
Flower	n-hexane	68

Table 5: Estimation of non–enzymatic antioxidant (Vitamin C)

Plant Part	Solvent extract	Vitamin C
Root	Aqueous	1.4 mg / g
Root	Methanol	1.2 mg /g
Root	n-hexane	1.7 mg / g
Fruit	Aqueous	2.5 mg/g
Fruit	Methanol	1.4 mg / g
Fruit	n-hexane	1.2 mg /g
Flower	Aqueous	1.9 mg / g
Flower	Methanol	1.1 mg / g
Flower	n-hexane	1.2 mg /g

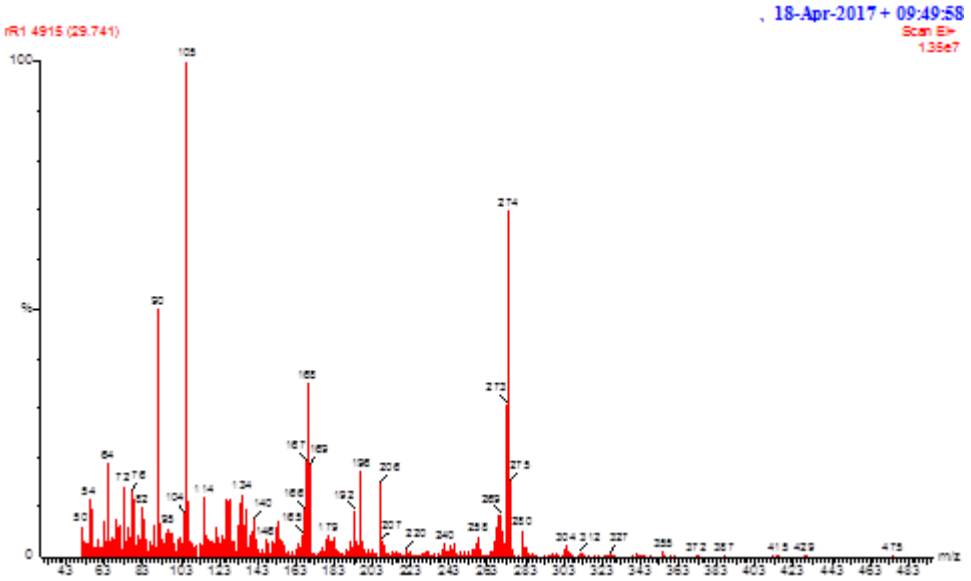

Fig. 1. GC-MS of Root extract(Methanol)

Table 6: Compounds identified in GC-MS analysis (Root Extract)

S.No	Compound name	Molecular weight	Molecular Formula
1.	2'-Phenylbenzanilide	273	$C^{19}H^{15}ON$
2.	Benzamide, N-(1,1'-biphenyl)-4-yl-	273	$C^{19}H^{15}ON$
3.	Benzamide, N,N-diphenyl-	273	$C^{19}H^{15}ON$

RESULT AND DISCUSSION

It is evident that the inhibitory activity was exhibited by the components present in *Berberis lycium* and not by the solvents used for extraction. From Table 1, it is clear that methanolic and aqueous extracts of flower followed by fruit and then followed by root have shown antimicrobial potential against selected stains. Flower extract is most potent against most of the selected bacterial

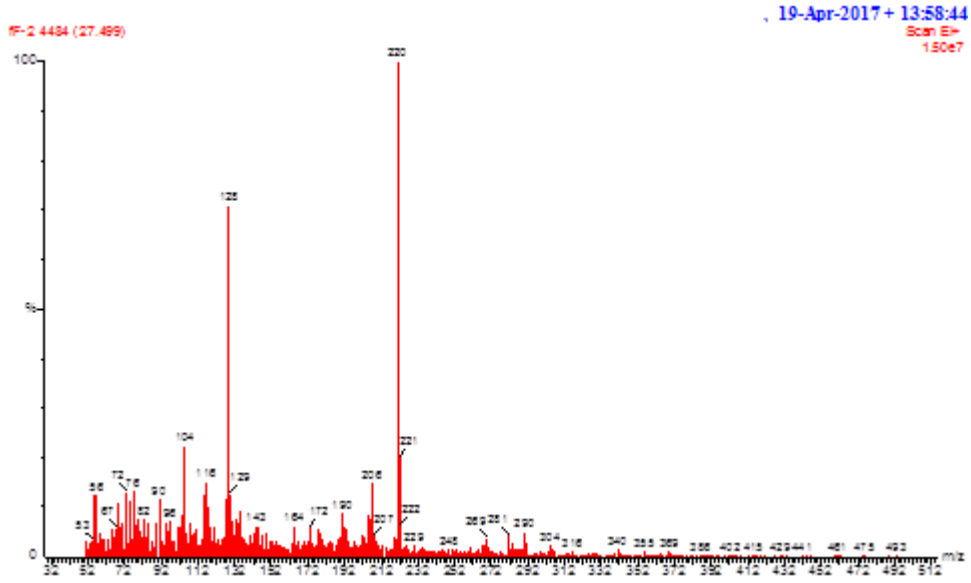

Fig. 2. GC-MS of Fruit Extract (Methanolic)

Table 7: Compounds identified in GC-MS analysis (Fruit Extract)

S.No	Compound name	Molecular weight	Molecular Formula
1.	Napthalene, 3-Benzyl-1, 2-Dihydro	220	$C^{17}H^{16}$

stains. Alcoholic extract of flower have shown maximum 28mm zone of inhibition against bacteria *Enterococcus* (*Gram positive*) followed by *Pseudomonas aeruginosa* (*Gram negative*) . In n-hexane extract it was found most potent against *Pseudomonas aeruginosa* and *Enterococcus* of 18 mm.

Aqueous extract of root have shown sensitivity against *salmonella* followed by

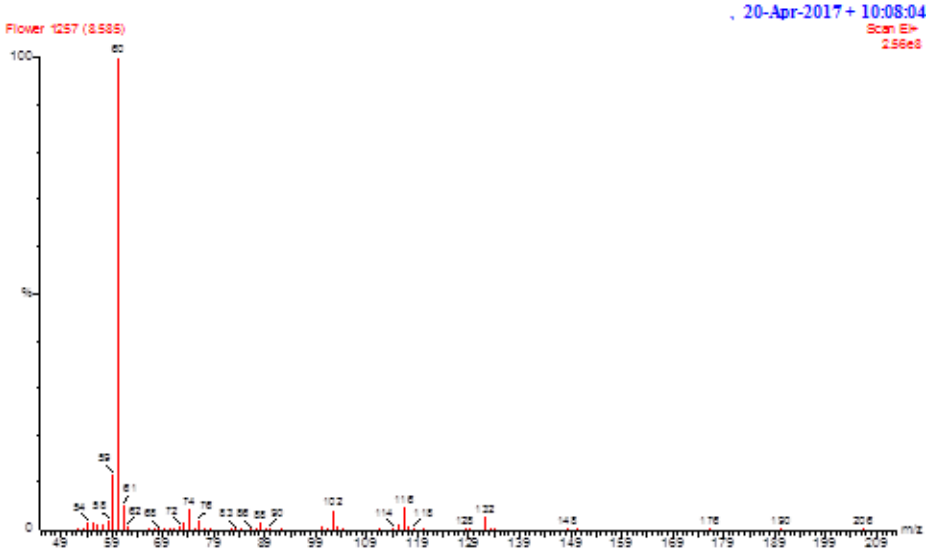

Fig. 3. GC-MS of Flower Extract (Methanolic)

Table 8: Compounds identified by GC-MS Analysis (Flower extract)

S.No	Compound Name	Molecular weight	Molecular Formula
1.	3(2H)-Thiophenone,Dihydro-2-Methyl	116	C^5H^8OS
2.	Butane,1-(Ethenylthio)-	116	$C^6H^{12}S$

Pseudomonas, similar trend of result was obtained by Ursoon Khan for anti-microbial effect of root extract of *Berberis lycium*[10]. Methanolic extract have shown maximum anti-microbial effect against *salmonella* (*Gram negative*) followed by *Acinetobacter baumannii* and *Klebsiella pneumoniae*. Alcoholic extract was not found effective against *Staphylococcus aureus* and *E.coli.*[8] reported that root extract not effective against *Staphylococcus aureus*, *Escherichia coli and Klebsiella.* Those results are almost similar for root extract with the finding in this study.

Aqueous fruit extract is effective against most of the bacteria selected and found to be most effective against *Pseudomonas aeruginosa* (*Gram negative*) with 16 mm zone of inhibition followed by 15 mm against *Enterococcus* (*Gram positive*). In this study it was observed that aqueous extract of fruit of *Berberis lyclum* is more potent than alcoholic fruit extract.

Flower extracts have shown antimicrobial effect against almost all bacterial strains, its flower extract was observed to be most effective against *Acinetobacter baumannii* (20 mm) followed by *Pseudomonas aeruginosa* (17 mm) and 16 mm against *Enterococcus.* Alcoholic extract of flower has shown maximum anti-microbial effect against *Enterococcus (28 mm)* followed by *Pseudomonas*

aeruginosa which is higher than shown by standard antibiotic(ciprofloxacin).[11] also studied antibacterial activity of root extract of this plant and results are almost in similar trend[11]. It can also be concluded that methanol is better solvent for antimicrobial activity of root extract similar to findings obtained by[7]. Overall in this study we observed strong antibacterial potential in flower extract followed by fruit and root extract. It was observed that the results of antibiotic properties almost in the similar trend[12]. It was found in their studied that berberine is main compound which is responsible for biological activity[12-13].

From Table 2 it is observed that flower extract is most potent extract against all the selected fungal strains while root extract was observed to be least effective . Alcoholic extract (flower) when tested against the fungal strains exhibited maximum inhibitory zone against *a.cuboida* followed by *A.niger* and *A.fumigatus* while Aqueous extract (flower) was observed to be almost ineffective. Fruit extract was observed to be effective against *A.niger*.

In this study investigating the *in vitro* antimicrobial activity, the results showed that the methanolic extract of flowers and fruit extract (methanolic extract) from *Berberis lycium* possessed maximum antibacterial activity in comparison to other extract which showed some or no activity, confirming the great potential of bioactive compounds .

Antioxidant activity has shown the presence of some antioxidant like vitamin C. Vitamin C is found to be highest in aqueous fruit extract 2.5 mg/g then followed by aqueous flower extract 1.9 mg/g showing the potential for future use. As shown in the Table 5. In Table 3. showed the presence of enzymatic antioxidant was 20.1 in aqueous fruit extract while 21.0 (unit/mg of protein) in methanolic fruit extract. 19.7 was found in n-hexane root extract.

Highest percentage of DPPH was found in n-hexane root extract followed by aqueous root extract that was 72 as shown in the Table 4.

Further when GC-MS of root, fruit and flower extract was done in which some bioactive compounds were identified. From Table 6 it was observed that these compounds were identified 2'-Phenylbenzanilide , Benzamide, N-(1,1'-Biphenyl)-4-YL-, Benzamide, N,N-Diphenyl. GC-MS analysis of methanolic fruit extract as shown in the Table 7 two compounds were identified 1-(4Methylphenyl)-4-Phenylbuta-1,3-diene and Napthalene, 3-benzyl-1,2-Dihydro. GC-MS flower methanolic extract showed the presence of 1,3,2-Oxathiaborole, 2-Ethyl-, 3(2H)-Thiophenone,Dihydro-2-Methyl and Butane, 1-(Ethenylthio)- as shown in the Table 8.

CONCLUSION

This means that some components are present in the root, fruit and flower of *Berberis lycium* that are responsible for the inhibitory activity of some of the bacterial and fungal strains. *In vivo* data may be helpful in determining the real potential usefulness of this plant for the treatment of infectious diseases. The medicinal plants have shown to have safety and efficacy in pharmacological activities including as antifungal activity.

The results showed that the flower, fruit and root extracts of *Berberis lycium* possessed good antimicrobial and antioxidant activity, confirming the great potential of bioactive compounds and are useful for rationalizing the use of this plant in primary health care. *In vivo* data may be helpful in determining the real potential of flower, fruit and root extracts of this plant.

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