Pharmacological, Antimicrobial, Antioxidant and Phytochemical analysis of folklore medicinal plant *Berberis lycium* L.

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Abstract

Berberis lyceum L. is a member of Berberidaceae traditionally used as food and folk medicines for the treatment of various diseases like cancer, diabetes, antioxidant and anti-microbial potential. In present study antimicrobial, antioxidant, analgesic and anti-spasmodic activities were taken under consideration *via*; *in vitro* and *in vivo* approaches. The results showed that the root extract of *Berberis lycium* L. ethanolic extract possess high amount of flavonoid as compared to phenols. Similarly, different phytochemical constituents like alkaloids, tannins, phlobatannins, flavonoids, carbohydrates, phenols, saponins, proteins and glycosides were found in the crude ethanol extract. The antioxidant activity of *Berberis lyceum* root was found to be the best in ethanol with percent potential was carried out at concentration of 0.05 mg/mL. The high percent potential of *Berberis lyceum* was observed in the ethanol extract 59.7% (at concentration of 0.05mg/mL) as compared to methanol (52.9%) and aqueous extract (28.5%). Similarly, different extracts of *Berberis lycium* was found effective in inhibiting the selected bacterial strains, however, methanol extract was found effective against all selected bacterial strains. In analgesic activity the highest writhing was count at concentration of 300mg/kg with 72% inhibition, followed by concentration of 200mg/kg showed 57.82%. The anti-spasmodic activity, at 300 mg/kg the charcoal meal transit was high and found effective. It is concluded from the present study that *Berberis lycium* L. has potential to treat different diseases ranging from anti-oxidant to

anti-microbial activities effectively and provide a base for isolation of specific compounds and use it on clinical trials.

Key words: analgesic, spasmodic, beberis, antioxidant, phytochemicals

1. INTRODUCTION

Medicinal plants have always played a key role in the life-span and approximately 80% of peoples in the developing countries still dependent on plants-derived drugs for the prevention of different disorders [1]. However, the usage of such plants needs proper method and knowledge depending on the nature of plants, part, method of remedies preparation, ecological zone of plants collection and disease condition [2]. Different plants have varied nature of phytochemicals and due to which the vast majority of structural variance among the compounds of the medicinal plants make them an excellent source for the novel therapeutics [4]. Around more than 10% of the 32,000 enlisted plant species are of medicinal importance globally. In developing countries different synthetic pharmacological drugs are expensive and not-affordable for the average economy family; consequently, plants are the primary source of phytomedicines for the pain, inflammation and microbial resistance [5]. Similarly, demand for the herbal medicine is increasing with passage of time that can ultimately can lead be met through screaming *via* different *in vitro* biological and *in vivo* approaches [6, 7].

Different biochemical and physiological processes occur in human bodies, due to which a number of free reactive oxygen species (ROS) are formed in the form of superoxide anion radical ($O2^{\cdot-}$), singlet oxygen ($^1O_2$), hydroxyl radical ($^{\cdot}OH$) and perhydroxyl radical ($HO^{2\cdot}$). One of the possible reasons for this problem is the use of much synthetic ingredients in the food which has increased the overproduction of ROS in the body. Due to which a number of chronic disorders like, cancer, diabetes, epilepsy, pain, cardiovascular and kidney problems. The ultimate and possible solution to this problem is the use of plants and plants derived products (medicinal herbs, fruits, seeds, flowers and vegetables) which contain precious phytochemicals like phenols which have the capabilities to scavenge such free radicles. Khan et al., [2021], recently reported that the intake of natural anti-oxidants significantly reduces the chances of different diseases in the human body.

Recently, it has been taken under consideration that important human health issues is getting sever, due to resistance caused by microbes against the now a days available drug and this is also serious tread that after 30years the existing drugs will be inactive against them. One of the ultimate sources

is the use of medicinal plants and their derivatives with least side effect as compared to synthetic drugs.

Berberis lycium is member of family berbidaceae commonly known as a Barberry and traditionally used for the treatment of different. The fruits of *B. lyceum* are sedative and also used as appetizer [5] and its bark is used in dysentery [6]. Studies have demonstrated a number of phytochemicals has been isolated from *Berberis sp.* e.g., protoberrines, berberine, isoquonoline etc. and have a number of medicinal properties like antibacterial, anti-inflammatory, antidiabetic and antimalarial [8]. Different phenolic compounds of berberry possess several pharmacological activities [9, 10, 11, 12]. *Berberis lycium* possess antiperiodic, antipyretic, antiulcer properties [13]. Similarly, different parts of the Berbaris are used to treat muscular pain, dirrohea and intestinal problems [14]. It is highly inhibiting ROS as antioxidant, effective in apoptosis and fighting against microorganisms.

The present study was carried with the aim to monitored different biological activities to investigate its potential capacities of different diseases being used traditionally. out to keep alive this traditional medicinal plant *Berberis lycium* L.

2. MATERIALS AND METHOD

2.1 Plant collection and processing

The roots of *Berberis lycium L.* were collected from Upper Dir, Khyber Pakhtunkhwa Pakistan. Plant was formerly identified by the professor at the Department of Botany, Abul Wali Khan University Mardan Pakistan. After identification the plant was also conformed in The Plant List (http://www.theplantlist.org/). On the same way, plant samples were properly washed, dried, crushed and extracted with ethanol solvent according to the standard procedure [16,17].

2.2 Phytochemical analysis

The qualitative phytochemical tests like, tannins, saponins, carbohydrates, alkaloids and flavonoids were carried out with protocols explained by Soni et al., [18].

2.2.1 Determination of total flavonoids

The total flavonoids quantification was done by taken 0.5g of *B. lyceum* root ethanolic extract. 0.1 mL potassium acetate was added and final volume was made up to 5mL. than the absorption was check at 415nm [19].

2.2.2 Determination of total phenols

The total Phenol quantification was done by mixing 0.5g plant sample to 1mL of 80% ethanol. Similarly, 2mL of 20% Na_2Co_3 solution was added. Then 0.5mL of folin-ciocalteau reagent was mixed well. Optical density was determined at a 650nm [20].

2.2 Biological activities

2.2.1 Antibacterial activity

Agar well diffusion method was used to carry out antibacterial activity using standard protocol of Pirzada et al., [21].

2.2.2 Antioxidant activity using DPPH as reducing agent

For the determination of DPPH 3.2µg DPPH was mixed in 25mL methanol and kept the solution at room temperature for 30 minutes. Similarly, 10µl of plant ethanolic extract were taken in different concentrations i.e., 0.05, 1 and 1.5mg/mL. to each extract of plant 90µl of DPPH solution was added. The mixtures were incubated for 30^{o}C for 30minutes. Absorbance was checked at 517nm [22].

Percent inhibition of DPPH Assay calculated by; 1As/Ab x 100.

2.3 Pharmacological activities

2.3.1 Analgesic activity

Acetic acid induced writhing test was used to check analgesic property of *B. lyceum*. Mice were grouped into five groups. Group 1st was treated with aspirin was used as standard and 2nd group was treated with standard drug Diclofenac sodium at a dose of 150mg/kg. Similarly, group3rd, 4th, and 5th were treated with ethanol extract at doses of 100, 200 and 300mg/kg, respectively. After one hour off the treatment, 20% v/v (10mL/kg) of acetic acid solution was administered by Intra peritoneal and writhes were counted for 15 minutes [23].

2.3.2 Anti-spasmodic activity

Charcoal meal was used to assess antispasmodic activity. Mice were grouped.into three different classes namely 1st, 2nd, 3rd, 4th and 5th. For solution of deactivated charcoal, 5gm charcoal was dissolved in 20mL distal water. Bascopan solution was used as standard and given to the 1st group.

Similarly, group 2nd was considered as a control (charcoal meal), while the rest group were treated with the plans ethanolic extracts at a concentration of 100, 200 and 300 mg/kg. After the preparation of solution, we have feed charcoal into the mice through feeding tube. After 20 minutes dissection of mice was carried out to measure the length of intestine in different group's low or high antispasmodic potential, following [24].

3. RESULTS

Qualitative phytochemical investigation of *Berberis lycium* L. was carried out for the presence or/and absence of saponins, carbohydrates, phenols, sterols, terpenoids, amino acid cordial glycoside and tannin in ethanol. It was taken under consideration that, maximum carbohydrates, amino acid, phenol, sterol and terpenoids were present, while tannin, saponins and cordial glycosides were completely absent in the ethanolic extract of *B. lyceum*.

Table.1 Qualitative phytochemical investigation of *Berberis lycium* L.

S. NO	Phytochemical tests	Ethanolic extract
1	Tannin	_
2	Saponins	_ _
3	Sterols	+++
4	Cordial glycosides	_
5	Terpenoids	++
6	Phenol	++
7	Amino acid	+++
8	Carbohydrates	+++

Here - = les quantity, -- = comparative quantity, ++ and +++ = more quantity

3.1 Quantitative investigation of flavonoids and total Phenolics

The root extract of the *Berberis lyceum* L. was studied for flavonoids and phenolic compounds in the solvents i.e., methanol, ethanol and aqueous (**Figure 1**). Maximum total flavonoid contents were found in the methanol extract (5.30±0.08), followed by ethanol extract (4.53±0.010µg/mL) by and aqueous extract showed least amount (3.50±0.04). Similarly, the methanolic extract showed total Phenolic contents of (3.44±0.05), followed by ethanol extract (2.30±1.22) and aqueous extract showed least amount (1.01±0.04) of total phenolic contents, respectively.

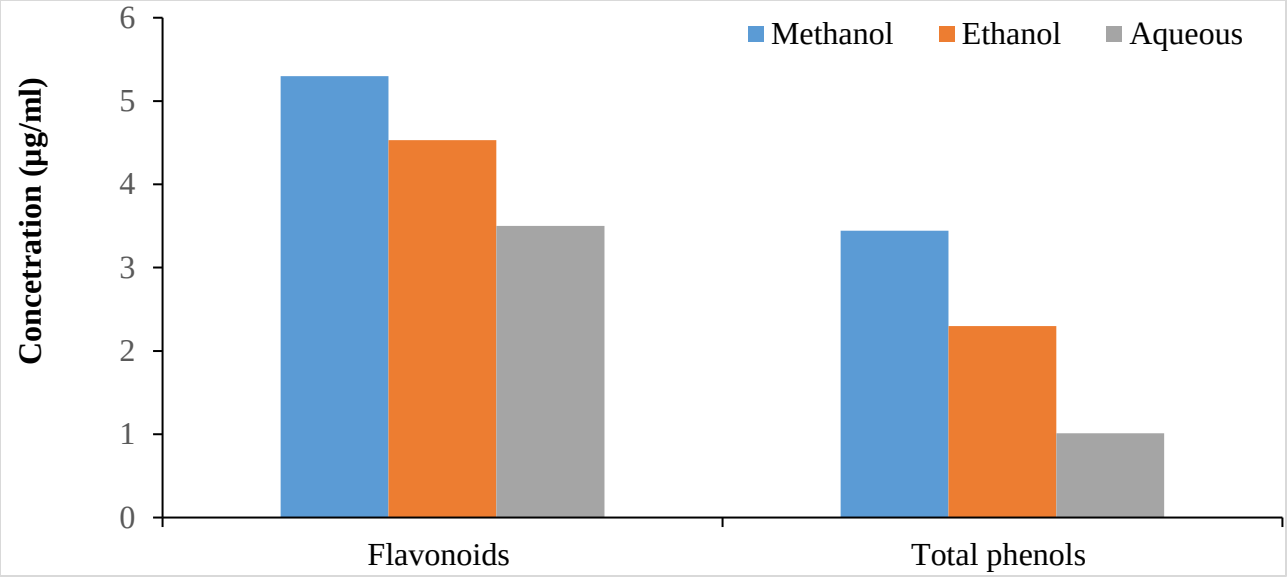


Figure 1. Total phenolic and Flavonoids contents in the root's extracts of *Berberis lyceum* L.

3.2 Antioxidant activity

The antioxidant activity of *Berberis lycium* L. was carried out for the methanol, ethanol and aqueous extracts of *Punica granatum*. Maximum, potential (59.7%) of antioxidant in *Berberis lycium* L. was observed in the ethanol extract, followed by methanol extract 52.9% and aqueous extract 28.58%, respectively. Similarly, at 1 mg/mL concentration high potential was observed for the ethanol extract 70% (0.42±0.001) followed by methanol extract 51.08% (0.48±0.11) and aqueous 39.7% (0.74±0.01), respectively. At maximum concentration i.e., 1.5 mg/mL the high percent potential was observed in ethanol extract 79% (0.41±0.07) followed by methanol extract 59% (0.44±0.159) and aqueous extract 43.9% (0.69±0.001).

Table.3 Antioxidant activity of *Berberis lyceum* L.

Concentration	Extracts	Mean±SD	%Potential
0.05 mg/ml	Methanol	0.41±0.04	52.9
	Ethanol	0.542±0.010	59.7
	Aqueous	0.84±0.004	28.58
1 mg/mL	Methanol	0.48±0.11	51.08
	Ethanol	0.42±0.001	70
	Aqueous	0.74±0.01	39.7
1.5 mg/mL	Methanol	0.44±0.159	59
	Ethanol	0.41±0.07	79

	Aqueous	0.69±0.001	43.9

3.3 Antibacterial activity

The antimicrobial activity of methanol, ethanol and aqueous extracts were found effective against selected bacterial strains (**Table 4**). The results showed that in case of *Staphylo coccus aureus* maximum inhibition was caused by aqueous extract (12.40±0.55), followed by methanol and ethanol extracts with 4.00±0.79 and 9.21±0.42 inhibitions, respectively. Similarly, *Shigella flexneri, Pseudomonas aeruginosa, Pseudomonas aeruginosa, Escherichia coli and Salmonella typhi* growth was inhibited by methanol extract with 13.80±0.61, 15.01± 0.45, 10.55± 0.77 and 19.80± 0.60% inhibitions, respectively.

Table 4. Antimicrobial activities of *Berberis lyceum* L.

Crude extract	***Staphylo coccus aureus***	***Shigella flexneri***	***Pseudomonas aeroginosa***	***Escherichia coli***	***Salmonella typhi***
Ethanol	4.00±0.79	6.80± 1.50	14.21± 0.20	4.40± 1.44	17.30± 0.40
Methanol	9.21±0.42	13.80±0.61	15.01± 0.45	10.55± 0.77	19.80± 0.60
Aqueous	12.40±0.55	8.88± 0.90	7.40± 0.71	9.00± 0.45	13.33± 0.44

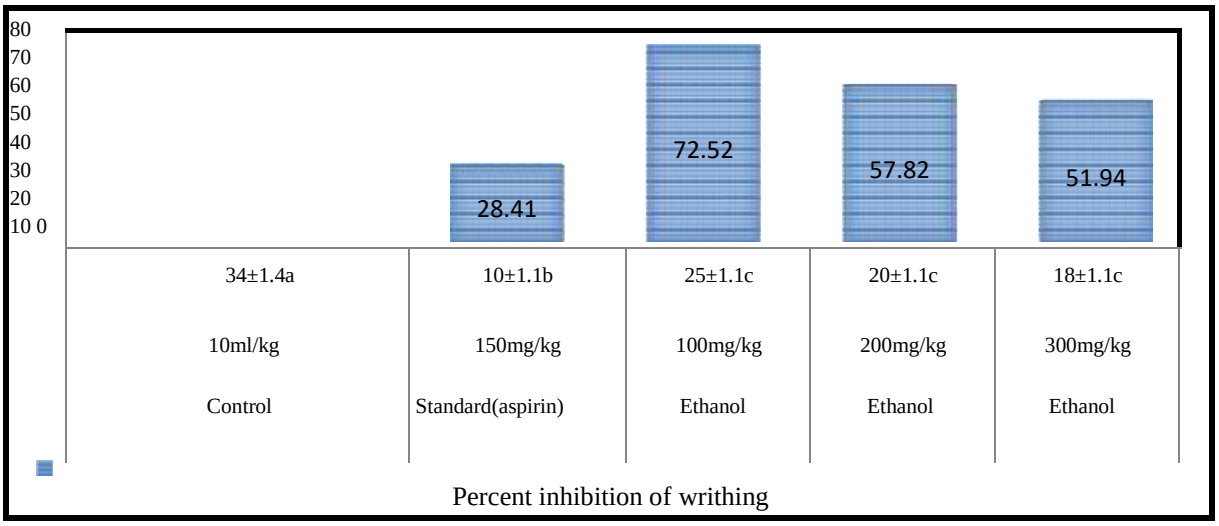
3.4 Analgesic activity

The mice of control group 1stshowed writhing i.e., 34±1.4^{a} /5 min. Group 2nd mice significantly reduced writhing i.e. 10±1.1^{b}/5 min and showed 28.41% inhibition. Among the group 3rd, 4th and 5th, the highest writhing counting was observed in Group 5th at the concentration of 300mg/kg (18±1.1^{c} in /5 min and showed 51% inhibition) followed by group 4th mice at a concentration of 200mg/kg (20±1.1) in 5 minutes and showed 57.82 inhibitions. Less number of writhing means high analgesic potential of the concentration (**Table 5**). It means that medicinal plant *Berberis lycium* has good analgesic effect at concentration of 300mg/kg.

Table.5 Analgesic activity of ethanolic extract of *Berberis lyceum.*

Groups	**Dose**	**number of writhing in 5 minutes mean± SEM**	**%age reduction of writhing**
Control	10ml/kg	34±1.4^{a}	

Standard (aspirin)	150mg/kg	10±1.1[b]	28.41
	100mg/kg	25±1.1[c]	72.52
Ethanol	200mg/kg	20±1.1[c]	57.82
Ethanol	300mg/kg	18±1.1[c]	51.94

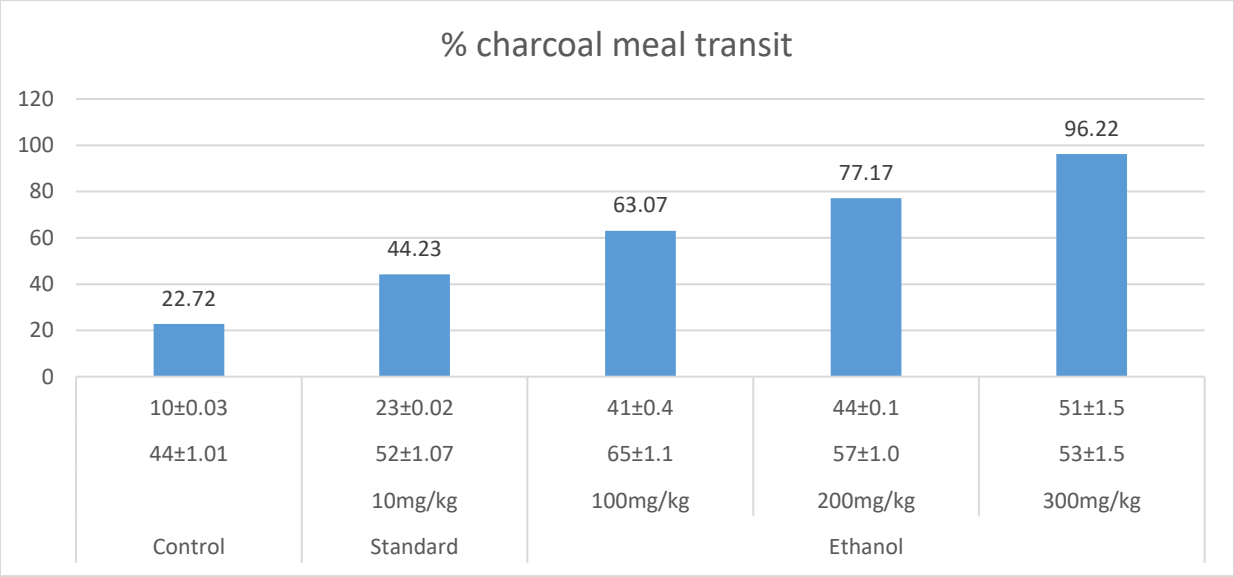


3.5 Anti-spasmodic activity

The mice of group 1st (control) were dissected and readings of total intestine length (cm) were noted (45.4±0.10). After feeding charcoal to mice of (G2 to G5) and left for 20 minutes. After 20 minutes' mice of all group were dissected and data were noted. The mice of G2 showed mean total intestinal length of 52±1.07, charcoal meal length was 23±0.02. The mice of group 3rdshowed mean total intestinal length of 65±1.07 and charcoal meal length is 41±0.04. The mice of group 4thhave mean total intestine length of 57±1.0 and charcoal meal length is 44±0.33. The mice of group 5thhave mean total intestine length of 53±1.5 and charcoal meal length is 51±0.4. The group 5th mice at the dose of 300mg/kg were noted as a significant result (21.5*±0.15), next was G4 with dose of300mg/kg (28*±0.02).

Table. 6 Anti-spasmodic activity of ethanol extracts of*Berberislycium*

Drugs	Dose mg/ml	Total intestine length(cm)	Charcoal meal length(cm)	% Charcoal meal transit
Control		44±1.01	10±0.03	22.72
Standard	10mg/kg	52±1.07	23±0.02	44.23
Ethanol	100mg/kg	65±1.1	41±0.4	63.07
	200mg/kg	57±1.0	44±0.1	77.17
	300mg/kg	53±1.5	51±1.5	96.22



4. DISCUSSION

The knowledge concerning the use of medicinal plants is unique and effective among the rural communities of Dir Khyber Pakhtunkhwa Pakistan [3]. As the plants like *Berbaris lyceum* L. is of key importance due to rich source of medicinal values for the treatment of different diseases. As *B. lyceum* is a woody plant so it is also used as fuel by the local communities. It is demand of the time to preserve its traditional knowledge and provoke its importance. Traditionally *Berberis lycium* L. is not only used for gastrointestinal problems, but it also cures muscular spasm. In the current study, pharmacological, phytochemical and biological activities on the root ethanolic extract of *Berberis lyceum* were checked, checked via; *in vitro* and *in vivo*.

Methanol extract was checked to analyze the pharmacological potential, while methanol, ethanol and aqueous extracts were used to analyze the phytochemical, antibacterial and antioxidant activity.

Flavonoid and phenols found in the plant make it a valuable for isolation of novel components which will be proven effective antioxidant in healing diseases. Phytochemical detection in methanol extracts showed positive result except saponins, and tennis. Similarly, total flavonoids and Phenol contents in methanol, ethanol and aqueous extracts were detected. The high amount of flavonoids contents was showed in methanol extract (5.26±0.50pg/mL followed by ethanol extract (4.61±0.50pg/mL) and aqueous extract showed lowest quantity (2.91±0.65pg/mL) while in methanol extract showed high quantity of phenol contents (6.35±0.581 pg./mL) compared with ethanol extract (5.00±0.988pg/mL) and aqueous extract showed lowest quantity. The methanol extract of *Berberis lyceum* given to worthy effects in analgesic and antispasmodic activities. In analgesic activity with dose of 300mg/kg the effect was significant (25±1.1) as compared to standard drug aspirin (10±1.1) writhing with 28.03% reduction. The Effective analgesic results with methanol extract were also presented by Iqbal et al., [25]. The reason could be due to the valuable compounds like berberine, protoberrines, isoquonoline of *Braberis lyceum* L. showed highly significant analgesic activity. Similarly, alkaloids reported from *Berberis lyceum* also showed analgesic, anti-spasmodic, anti-malarial and antibacterial activities [26-28].

In antispasmodic studies, at a dose 300mg/kg showed (53±1.1) and 49.76% significant inhibition followed by the dose of 100mg/kg (65±1.5) and 63.34% and 200mg/kg (57±1.53) and 59.56% inhibition, compared with standard drug atropine (52±1.05 and the inhibition (78.71%). Recently, Rehman et al., [29] reported the spasmogenic effects in isolated rabbit jejunum and ileum. It is suggested that the plant *Berberis lycium* L. possess atropine which for the time being suppressed the contraction of muscles. Other possible reason could be due to the presence of flavonoids in various forms; such as quercetin, genistein, naringenin and apigenin can normalized gastrointestinal irregularities based on the internal mechanisms involved [30]. Similarly, study of Ragone et al., [31], reported that duodenum spams can be control with vitexin type of flavonoid. Some compounds of flavonoids have been reported which are not only anti-spasmodic but also causal agents of such twinges which includes triggering of special receptor like muscarinic-receptors and in the former blockage of calcium canals [32; 33]. Similarly, other studies reported

that flavonoids isolated from the plant are of anti-spasmodic properties while consumption of phenols of the same plant have properties to produce intestinal cramps [34]. Many reports are there to justify that the constituents of flavonoids possess positive effects of analgesic, anti-spasmodic, antibacterial anticancer and many more activities [35].

5. CONCLUSION

The present study demonstrated that plant are massive sites of healing and would be help full in introducing specific and safe drugs. In the present study different phytochemical qualitative and quantitative were taken under consideration. Similarly, the *Berberis lycium* L. possesses significant positive effects on the anti-bacterial activities studied conforming the presence of enormous phytochemicals in the plant. Further research in needed for the isolation and identification of bioactive compounds and to contribute novelty to drugs discovery, *via*; clinical trials.

6. CONFLICT OF INTEREST

Authors have no conflict of interest.

7. Author's contribution

All authors contributed equally for the successful completion of this project.

8. ETHICAL APPROVAL

All experiment has been examined and approved by the ethical committee.**9. REFERENCES**

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