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RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS, ANALGESIC, ANTI-INFLAMMATORY AND ANTI BACTERIAL ACTIVITIES OF *BERBERIS LYCIUM.*

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Abstract

In the present research work the phytochemical investigation of methanolic, ethanolic and chloroform extracts of*Berberis lycium* and pharmacological activities of methanolic extracts (Anti-inflammatory and analgesic activity) and antibacterialactivities in methanolic, ethanolic and chloroform extracts was carried out. The phytochemicals analysis showing the presence ofcarbohydrates, flavonoids, phlobatannins, alkaloids, saponins, tannins, phenols, terpenoids, cardiac glycosides was present in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides and protein were absent and quantative phytochemistry showed the flavonoids in chloroform extract as (14.20±0.15mg/ml), Alkaloids (12.10±0.15mg/ml), phenolics (10.45± 0.10mg/ml), Saponins (06.22±0.14mg/ml) and Tannins (04.60±0.65 mg/ml). The pharmacological activities such as Anti-inflammatory, at the doses of 600 mg/kg b.w the administration of extract produced a significant anti-inflammatory activity at 3 hours with paw oedema inhibition of 59 % respectively, while the standard drug aspirin inhibited paw oedema of 68%. In analgesic activity the results showed significant dose dependent % inhibition of pain responses in extract 600mg/Kg is 61%. In antibacterial activity the most active among the extracts was with (17.00±0.48 mm) zone of inhibition at the concentration of18 mg/µl against *Pseudomonas aeroginosa*. Fallowed by *Escherchia coli*(16.27±0.93mm), *Shigella flexneri*(16.20±1.89mm) and *Salmonella typhi*(16.11± 0.82) with concentration of 12 mg/µl.

Introduction:-

BibliographyBibliography:-

Plants are contain various phytochemical constituents such as tannins, flavonoids terpenoids, phenolic acids, vitamins,lignins, quinines, stilbenes, amines, coumarins, betalains, alkaloids, and other phytochemicals, which have the potential of antioxidant activity (Zheng and wang, 2001). Research have showed that several of these antioxidant compounds have ant atherosclerotic, anti-inflammatory,anti-mutagenic, antitumor,antifungal, antiviral and anti-carcinogenic activities (Ganesan *et al*., 2017). In modern research, plant phytochemicals, formerly with unknown and biological activities, have been widelystudied as a basis of medicinal agents (Krishnaraju *et al*., 2015).

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Therefore, it is estimated that plants phytochemicals with sufficientantifungalability will be used for the cure of bacterial infections (Gracelin *et al*., 2013). Pain may be define "unpleasant sensory and emotional experience that is caused by actual or potential tissue damage". The emotional component differs from one person to the other and in the same individual from time to time and it can be classified in several ways, but in therapeutic application into; nociceptive and neuropathic (Koech *et al*., 2017). In the body, Sensory nerve endings are generally found in every part of the body such as the blood vessels, internal organs, muscles, joints, and the skin (Thorp, 2008). In the brain, pain stimulus is processed and generated impulses are send down the spinal cord following the appropriate nerves and instructs the body to respond, for instance withdrawing your hand from the fire (Rang *et al*.,2006).Inflammation is the complex biological response of vascular tissues to harmful stimuli including irritants, pathogens, or damaged cells. It is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue (Denko *et al.,* 1992). The process of inflammation is necessary in healing of wounds. Inflammation however, if runs unchecked, lead to onset of diseases like vasomotor rhinnorhoea, rheumatoid arthritis and atherosclerosis (Savill *et al.,* 1989). Acute inflammation is characterised by classical signs edema, erythrema, pain, heat, and above all, loss of function. The classical signs are triggered by the infiltration of the tissues by serum and white blood corpuscles (leucocytes). Chronic inflammation results in a progressive shift in type of cells, present at site of inflammation. It is characterized by simultaneous destruction and healing of the injured tissue from incidence of inflammation (Janaranjani *et al*., 2014). Fever or pyretic is defined as the elevation of core body temperature above normal; in normal adults, the average oral temperature is 36.98C (98.58F). In oncology practice, a single temperature of more than 38.3°C (101°F) or three readings (at least 1 hour apart) of more than 38°C (100.4°F) are considered significant. Lower temperature elevations in the very young or old and in patients receiving steroids or other immune suppressants are considered abnormal (Mackowiak *et al*., 1997).*Berberis lycium* belonging to family Berberidaceae is a high-value medicinal plant with a known history of uses in folk medicine. It is used traditionally for curing a broad range of human illnesses and diseases in the Indian Himalayan region of Pakistan and India. Its ethno medicinal uses include its use for treatment of jaundice, diabetes, eye infections, fractured bones, internal wounds, diarrhea, rheumatism, stomachache, and its use as a general body tonic (Ali *et al*., 2015).

Material and Methods:-

Collection of plant materials and Botanical Identification:-

In the present study, *Berberis lycium* was collected in March, 2017 from Garrah Districtlower Dir, of Khyber Pakhtunkhwa Province Pakistan. With the help of Flora of Pakistan plant were taxonomically identified and placed in the Herbarium of Abdul Wali Khan University Mardan Garden campus.

Solvents:-

For the crude extract preparation of the *Berberis lycium* methanol, ethanol and chloroform was used.

Crashing and filtration of the plant:-

The dried plant was powdered with the help of electric grinder. The powder were kept in air tight plastic bottles for further phytochemical, nutritional analysis, pharmacological and antifungal activities. 15 gm of plant powdered was retained in distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol and chloroform) was added to the powdered separately. With the help of aluminum foil the flask were covered and retained in shaker for 72 hrs for the shaking purposes. After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through filtration process plant extracts were removed (Pirzada *et al.,* 2010).

Phytochemical Analysis:-

Qualitative study:-

The plant extract i.e. methanol, ethanol and chloroform were tasted for the absence or presence of phytochemical constituents' like alkaloids, tannins ,Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides and terpenoids (Soni *et al.,* 2011) .

Tests for Alkaloids:-

For detection of alkaloids, a few drops of Wagner's reagent (Potassium iodine) are add to 2 ml of all three methanol, ethanol and aqouse extracts. The presence of alkaloids was confirmed by the formation of reddish brown precipitate (Khandewal *et al.,* 2015).

Tests forTannins:-
For the detection of tannins Ferric chloride test was done. Ferric chloride ($FeCl_3$) solution was mixed with all three extracts separately. Formation of blue green coloration indicated the presence of tannins. (Kokate *et al*., 2008).

Tests for Phlobatannins:-
In test tubes 0.5 ml of all the three extracts was taken separately, added 3ml distilled water and shaken for a few minutes then 1% aqueous hydro chloride (HCl) was added and boiled on water both. The presence of phlobatannins is indicated by the formation of red color (Wadood *et al.,* 2013).

Tests for Flavonoids:-
For flavonoids detection, sodium hydroxide (NaOH) solution was added to all the three extracts of the plant. Red precipitation formation of indicate the presence of flavonoids (Kokate *et al.,* 2008).

Tests for Carbohydrates:-
For detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's regent.Solution were heated for 2 minutes on a water bath. By the formation of reddish brown precipitate the presence of carbohydrate was confirmed (Bussau *et al*., 2002).

Tests for Phenols:-
For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml ofall the threeextracts separately in a test tube. Deep bluish green colorationformations indicated the presence of phenol (Dahiru *et al.,* 2006).

Tests for Saponins:-
For the detection of saponin, in test tube 5 ml of all three extracts were shaken vigorously. the presence of saponins was confirmed by froth formation (Rajesh *et al*.,2016).

Tests for (Cardiac) Glycosides:-
For cardiac glycosides detection, 2 ml of all three extracts solution were shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid (H_2SO4) and iron tri chloride ($FeCl_3$). Brown ring formation indicated the presence of Cardiac glycosides (Soni *et al.,* 2011).

Tests for Proteins:-
Xanthoproteic test: For the detection of protein1 ml from of all three extracts were treated with 1ml of concentrated nitric acid (HNO_3) solution. The presence of proteins indicated by the formation of yellow color (Rajesh *et al.,* 2016).

Tests for Terpenoids:-
salkowski test:- One ml of plant extracts (methanol, ethanol and chloroform) was added with 2 ml of chloroform and carefully added concentrated sulphuric acid (H_2SO_4) along the sides of tube to form a layer.The reddish brown coloration formation indicated the presence of terpenoids (Dahiru *et al.,* 2006).

Tests for Glycosides:-
For the detection of glycosides, 5% of Ferric chloride solution and 1 ml glacial acetic acid were added to 5 ml of all three extracts and then further addition of few drops of concentered sulphuric acid (H_2SO_4). The presence of glycosides was conformed through the formation of greenish blue color (Rajesh *et al.,* 2016).

Quantitative Analysis:-
Determination of total flavonoids contents:-
Ethanol, methanol and chloroform extracts were used for the detection of total flavonoids contents. Total flavonoids quantification was done by taking 0.5 g of plant extracts. Than the sample were mixed with 4.3 ml methanol and then more addition of 0.1 ml of aluminum tri chloride from 10% prepared solutions of aluminum tri chloride laterally. Potassium acetate (0.1 ml) was added the volume was reached to 5 ml. The mixtures were shaken by vortex to make uniform solution and then these mixture were placed at room temperature for 30 minutes for the purpose of incubation. After the completion of incubation process, the absorption was checked at 415 nm in spectrum. The Quercetin was used as a standard (Daffodil *et al* ., 2013).

Determination of Total Phenolic Contents:-
Total phenolic quantification was done by the addition of 0.5g plant extract to 1 ml of 80% ethanol. Then the mixture were centrifuged for 15 minutes at 12,000 rpm. After that the supernatant were kept in test tube and these process were repeated 6 times. After collecting the supernatant were placed in water bath for drying. The distilled water was added to the supernatant until its volume reached to 3 ml. 2 ml (Na_2CO_3) of 20% were added in this solution. To this 0.5 ml Folin -ciocalteau regent was added and after 5 minutes more addition of 2 ml (Na_2Co_3) from 20% Na_2Co_3 solutions. The solution were mixed homogenously and then the test tube were brought in to the water bath in boiling water. At 650 nm their absorbance were checked. The Catechol was used as a standard (Gracelin *et al.,* 2013).

Quantification of total alkaloids:-
5 gm of the all the three extracts was weighed in a 250 ml beaker and 200 ml of 10% acetic acid in ethanol was added and covered than allowed for 4 hours to stand. Extracts was filtered and was concentrated on a water bath to one-quarter of the original volume. Until the precipitation was completeDrop wise to the extract concentrated ammonium hydroxide was added. The solution was allowed to settle and collected the precipitated and washed with dilute ammonium hydroxide and then filtered. The residue was the alkaloid, was dried and weighed (Gracelin *et al.,* 2013).

Determination of total tannins:-
500 mg of the sample was weighed in a 50 ml plastic bottle. Added 50 ml of distilled water and shacked for 1 hours in a mechanical shaker. In a 50 ml volumetric flask was filtered and made up to the mark. Into a test tube, 5 ml of the filtered was pipetted out and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium Ferro cyanide. At 120 nmtheir absorbance was cheeked out (Gracelin *et al*., 2013).

Determination of total saponins:-
Into a conical flask 20 gm of each extracts were put and 100 cm3 of 20% ethanol, aqueous were added. Over a hot water bath for 4 hours the samples were heated with continuous stirring at about 55°C. The residue re-extracted with another 200 ml 20% ethanol, and mixture was filtered. At about 90°C through water bath the combined extracts were reduced to 40 ml. Into a 250 ml separatory funnel the concentrate was transferred and 20 ml of diethyl ether was added and vigorously shaken. 60 ml of n-butanol was added. With 10 ml of 5% aqueous sodium chloride the combined n-butanol extracts were washed twice. In a water bath the remaining solution was heated. The samples were dried in the oven to a constant weight after evaporation; the saponin content was calculated (Gracelin *et al*., 2013).

Pharmacological activities:-
Pharmacological activities was carried out in methanolic extracts of *Berberis lycium* plant.

Experimental animals:-
Both sexes of the albino mice of weight about 25 - 30 gm were brought from the animal house of National Institute of Health (NIH), Islamabad. The animals were supplied with adlibitum water and standard pellet diet.

Drugs used and chemicals used:-
Aspirin (Bayer, Germany), were used as standard drugs in the experiment for selected activities. Methanol 95% (Merck, Germany), Normal saline (Immunasol NS, A.Z. Pharmaceuticals Co.pak), carrageenan (Lewis Labs, U.S) and Acetic Acid (Lewis Labs, U.S).

Anti-inflammatory activity by carrageenan induced inflammation:-
The mice were divided into five groups. Groups 3rd, 4th and 5th were treated with methanolic extract of at the doses of 200, 400 and 600mg/Kg respectively. Mice in the group 1 were given normal saline 10ml/kg. The 2nd group was treated with standard anti-inflammatory drug Aspirin(75mg) 150mg/kg. Carrageenan (20%) was injected in the right hind paw of albino mice of group 2nd, 3rd, 4th and 5th. Carrageenan solution was prepared by dissolving 20% carrageenan in 80% distilled water. To the mice of group 3rd, 4th and 5th. Methanolic extract at the dose of 200, 400 and 600mg/Kg was injected respectively in to the hind paw checked the diameter. Left for some time after extract injection and then measured the paw diameter at 1hour interval up to 4 hours and was compared with standard drugs to note the low and high potential of inflammation (Akhter *et al*., 2009).

Evaluation of Analgesic Activity:-

Acetic acid induced writhing test:-

The mice were distributed into five groups. Groups 3rd, 4th and 5th were treated with methanolic extract of*Berberis lycium* at the doses of 200, 400 and 600mg/Kg respectively. The 2nd group was treated with standard drug aspirin 150mg/kg. One hour after the treatment, 20% (10ml/kg) of acetic acid solution was injected by intra peritoneal injection. Abdominal writhings were counted for 5 minutes. For analgesic activity 1cc acetic acid (20%) was injected in to group 2nd, 3rd, 4th and 5th of mice. Aspirin solutions was prepared by dissolving one tablet in 10 ml of water. The solution was injected into mice of group 2nd and checked the number of writhing's per 5 minutes. To the mice of group 3rd, 4th and 5th methanolic extract at the dose of 200, 400 and 600mg/Kg was injected respectively. The results of group 3rd, 4th and 5th was compared to the group 2nd for low or high analgesic potential. The % inhibition calculated by the following formula (Akhter *et al*., 2009).

$$\% \text{ inhibition} = 1 + \frac{No.ofwrithingsintesteddrug}{No.ofwrithingsincontrol} \times 100$$

Antibacterial Activity

Crude extracts Antibacterial:-

To screen the antifungal activity of the selected medicinal plants chloroform, methanolic and ethanolic extracts the agar well diffusion method was used (Perez *et al*., 1990). With all crude extracts of plants the assay was performed.

Microorganisms used:-

For antifungal activity *Pseudomonas aeruginosa,Escherichia coli, Shigella flexneri* and *Salmonella typhi, (*Gram-negative), *Staphylococcus aureus* (Gram-positive) bacteria were used.

Media for bacterial culture:-

By dissolving 25 g/l in distilled water Luria Broth, miller medium was prepared. Media PH was adjusted to 7.0. 100 ml of LB broth was distributed in 250 ml flask and autoclaved. In flasks Bacterial strains were inoculated and kept at 37ºC in shaker incubator at 150rpm overnight. By dissolving 40 g of LB agar in 1 liter of distilled water LB Agar was prepared and pH was adjusted and autoclaved.

Inoculum Preparation:-

Bacteria strains from 24-hour old culture in LB broth (Miller) of selected bacterial strains were mixed with physiological normal saline solution until a McFarland turbidity standard [10^6 colony forming unit (CFU) ml^{-1}] was obtained. In LB Agar Medium then this inoculum was used to seed.

Agar PlatesPreparation:-

At room temperature LB agar was left to cool, it was poured into sterilized petri plates before to solidify.The agar well diffusion method (Perez *et al*., 1990) was used. Using a sterile cotton swab cultures lawn of the test organisms were made on the agar plates.Using a sterile borer under sterile conditions five wells were made per plate.

Extract Preparation for activity:-

In 1 ml of DMSO 20mg crude extracts of all plant samples were completely dissolved. Solution of a standard antibiotic (2 mg/ml of Cefotaxime) was used as positive control. Negative control was used pure DMSO.

Measurement of zone of inhibition and Pouring of test solution incubation:-

Using micropipette, 75 µl of plant samples solution were poured in labeled wells. Each of the labeled plate was provided with samples of extracts, as positive standard cefotaxime and as negative standard di methyl sulphoxide (DMSO) was used. At 37ºC incubation was done. After 24 h of incubation, the diameter of clear zones, showing no bacterial growth around each well was measured. Three times activity was repeated and average of zone of inhibition with standard deviation was calculated.

Statistical analysis:-

All the tests were performed as individual triplicate experiment. All the data are shown as mean ± standard error of mean (S.E.M., *n* = number of Experiments). The statistical analyses were obtained by the one way analysis of variance (ANOVA), followed by the Dennett's test where necessary. $P<0.05$ was considered Significant.

Result and Discussion:-

Phytochemical analysis:-

In the present research work the phytochemical investigation of methanolic, ethanolic and chloroform extracts of*Berberis lycium* and Pharmacological activities of methanolic extracts (Anti-inflammatory, analgesic activity) and antibacterial activities in methanolic, ethanolic and chloroform extracts was carried out.

Phytochemical detection in the roots of*Berberis lyceum:-*

Qualitative analysis of *Berberis lycium* was carried out for the detection ofalkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides and proteins. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides and protein were found absent in the chloroform extracts. Flavonoids, carbohydrates, saponins, phenols and terpenoids were found present in in the rhizome methanolic and ethanolic extracts. In these results +++ indicate that the secondary metabolites present in highest amount, the ++ indicated that the moderate level of phytochemicals' are present and the + indicated that low level of phytochemicals are present and – indicated that the phytochemicals are absent in all these three extracts plants (Table 1, 2).

Table 1:-Qualitative Detection of Bioactive compound in the roots of *Berberis lycium* in methanolic, ethanolic and chloroform extracts

S.NO	Phytochemical test	Methanolic	Ethanolic	chloroform
1	Alkaloid	+++	++	_
2	Flavonoids	+	+++	+
3	Carbohydrate	+++	+	+
4	Phlobatannins	+++	++	_
5	Glycosides	+	+++	+
6	Saponins	+	++	+
7	Phenol	+++	++	+
8	Terpenoids	++	+++	_
9	Tannins	+++	++	+
10	Cardiac glycosides	++	+	_
11	Proteins	++	+	_

Key: +++: present highest level, ++ showed moderate level, + showed low level - absent

Table 2:-Phytochemicals detection in the leaves of *Berberis lycium* in methanolic, ethanolic andchloroform extracts

S.NO	Phytochemical test	Methanolic	Ethanolic	chloroform
1	Alkaloid	++	+	_
2	Flavonoids	+	+++	+
3	Carbohydrate	+++	++	+
4	Phlobatannins	+++	++	_
5	Glycosides	++	+++	_
6	Saponins	+++	+++	+
7	Phenol	+++	+	+
8	Terpenoids	++	+++	+
9	Tannins	+++	++	+
10	Cardiac glycosides	++	+++	_
11	Proteins	++	++	_

Key: +++: present highest level, ++ showed moderate level, + showed low level - absent

Total Phenolic, Flavonoids, Tannins,Saponins andAlkaloids Contents in chloroform, methanol and ethanol:-

Highest amount of flavonoids was found in the chloroform extract as (14.20 ± 0.15mg/ml) followed by Alkaloids (12.10 ± 0.15mg/ml), phenolics (10.45 ± 0.10mg/ml), Saponins (06.22 ± 0.14mg/ml) and lowest amount of Tannins

was found in (04.60 ± 0.65 mg/ml). The flavonoids was found in highest amount in methanolic as (17.55 ± 0.10mg/ml), followed by phenols (13.25 ± 0.50mg/ml), Tannins (11.55 ± 0.30mg/ml), Alkaloids (10.05 ± 0.10mg/ml) and Saponins was found in lowest amount (08.40 ± 0.45 mg/ml). The flavonoids was found in highest amount in methanolic as (13.25 ± 0.50 mg/ml), followed by phenols (11.25 ± 0.10 mg/ml), Alkaloids (09.50 ± 0.15 mg/ml), Tannins (06.25 ± 0.40 mg/ml) and Saponins was found in lowest amount (05.40 ± 0.25 mg/ml). The data is showed in (Table 3, 4 and 5).

Table3:-Qualitative Phytochemistry of *Berberis lycium* in chloroform extracts

S.No	**Phytochemicals name**	**Concentration mg/ml**
1	Total phenolics	10.45 ± 0.10
2	Total flavonoids	14.20 ± 0.15
4	Total Tannins	04.60 ± 0.65
5	Total Saponins	06.22 ± 0.14
6	Total Alkaloids	12.10 ± 0.15

Table4:-Qualitative Phytochemistry of *Berberis lycium* in methanolic extracts

S.No	Phytochemicals name	Concentration mg/ml
1	Total phenolics	13.25 ± 0.50
2	Total flavonoids	17.55 ± 0.10
4	Total Tannins	11.55 ± 0.30
5	Total Saponins	08.40 ± 0.45
6	Total Alkaloids	10.05 ± 0.10

Table5:-Qualitative Phytochemistry of *Berberis lycium* in ethanolic extracts

S.No	Phytochemicals name	Concentration mg/ml
1	Total phenolics	11.25 ± 0.10
2	Total flavonoids	13.25 ± 0.50
4	Total Tannins	06.25 ± 0.40
5	Total Saponins	05.40 ± 0.25
6	Total Alkaloids	09.50 ± 0.15

Pharmacological activities:-

For pharmacological activities methanolic extracts was used.

Anti-inflammatory activity:-

The effect of *Berberis lycium* whole plant on carrageenan induced paw oedema is shown in the table 1. After injection of carrageenan the albino mice paw become oedemateous. At the doses of 200,400,600 mg/kg b.w the administration of extract produced a significant anti-inflammatory activity at 3 hours with paw oedema inhibition of 28%, 41 % and 59 % respectively, while the standard drug aspirin inhibited paw oedema of 68%. When compared with standard drug at the dose of 600 mg/Kg the extract showed a maximum inhibition of carrageenan induced mice paw oedema. Data showed in table (6).

Table6:-Anti-inflammatory activity of *Berberis lycium* whole plant methanolic extracts

Mice hind paw size (mm)							
Drug	**Dose**	**Before inflammation (mean ± SEM)**	**Afterinflammation (mean ± SEM)**	**1 hour (mean ± SEM)**	**2 hours (mean ± SEM)**	**3 hours (mean ± SEM)**	**Percentage (%) of inhibition**
control	N/S	1.13±0.21	1.42±0.12	1.91±0.49	2.47±0.62	2.68±0.17	……..
Aspirin	150mg/Kg	1.07±0.15	2.8. ±0.16	5.8±0.14	2.2±0.22	5.7±0.27	68%
Methanolic extract	200mg/Kg	0.64. ±0.21	1.4. ±0.12	3.9±0.17	3.1±0.62	2.9±0.49	28%
Methanoli	400mg/K	2.7. ±0.18	2.6±0.08	5.4±0.41	7.3±0.22	7.1±0.12	41%

c extract	g						
Methanolic extract	600mg/Kg	2.2. ±0.12	7.6±0.16	7.4±0.17	7.6±0.20	7.9±0.13	59%

Analgesic activity (Acetic acid induced writhing test):-

The methanolic extract of *Berberis lycium* used injected at different doses (200, 400 and 600mg/Kg) showed significant and dose dependent inhibition pain responses. The number of writhings in extract 200mg/Kg was 60.6±0.245 in 5 minutes, 400mg/Kg 45.2±0.2 and in extract 600mg/Kg was 40.2±0.374*. the results showed significant dose dependent % inhibition of pain responses in extract 200 mg/Kg 17%, in extract 400 mg/Kg is 31% and in extract 600mg/Kg is 61%. The percentage inhibition of pain by 150mg/Kg aspirin was found to be 78% as compared to the other doses of the *Pteris quadriaurita*. The data is shown in table (Table 7).

Table7:-Effect of methanolic extracts of *Berberis lycium* in Acetic acid induced writhings test

Drugs	**Dose mg/Kg**	**Number of writhings in 5 minutes (mean± SEM)**	**% Inhibition of writhings**
N/S	10ml/Kg	80.4±0.245	…….
Aspirin	150mg/Kg	39.2±0.2	73%
Methanolic extract	200mg/Kg	60.6±0.245	17%
Methanolic extract	400mg/Kg	45.2±0.2	31%
Methanolic extract	600mg/Kg	40.2±0.374*	61%

Antifungal activity of methanolic, ethanolic and chloroform extracts of*Berberis lycium* against selected fungal strains:-

The results of antifungal activity showed that Crude methanolic Extract were active against all fungal species and showed different range of zone of inhibition. The most active among theextracts was with (17.00± 0.48mm) zone of inhibition at the concentration of18 mg/µl against *Pseudomonas aeroginosa*. Fallowed by *Escherchia coli*(16.27±0.93mm), *Shigella flexneri*(16.20± 1.89mm) and *Salmonella typhi* (16.11± 0.82) with concentration of 12 mg/µl. And the ethanolic extracts showed maximum zone of inhibition (16.06± 0.97 mm) at the concentration 12 mg/µl against *Salmonella typhi*fallowed by *Shigella flexneri*(15.33± 1.12 mm) at the concentration of 6 mg/µl and lowest amount of inhibition was showed against *Pseudomonas aeroginosa*(11.13±1.65mm) and *Salmonella typhi* (10.062±1.32mm). The chloroform extracts showed maximum inhibition against *Pseudomonas aeroginosa*(16.63± 0.65mm) at the concentration of12 mg/µl, followed by *Salmonella typhi*(16.18± 1.43mm) at the concentration of 6 mg/µl,*Staphylococcus aureus*(*14.40± 1.34*) at the concentration of 12 mg/µl and lowest amount of inhibition was showed *Shigella flexneri*(10.23± 0.88mm), *Escherchia coli* (10.00± 3.60mm) and *Staphylococcus aureus*(8.56± 1.75mm) at the concentration of 18 mg/µl and 6 mg/µl.The data are shown in table (8, 9 and 10).

Table8:-Antifungal activity of chloroform extracts of *Berberis lycium* against selected fungal strains

Extracts concentration	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeroginosa*	*Escherchia coli*	*Salmonella typhi*
6 mg/µl	8.56± 1.75	13.37± 1.52	9.83± 0.46	12.33± 0.76	16.18± 1.43
12 mg/µl	14.40± 1.34	10.47± 0.67	16.63± 0.65	13.47± 1.32	14.09± 0.87
18 mg/µl	11.00± 1.20	10.23± 0.88	12.37± 1.86	10.00± 3.60	12.00± 2.08

Table9:-Antifungal activity of ethanolic extracts of *Berberis lycium* against selected fungal strains

Extracts concentration	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeroginosa*	*Escherchia coli*	*Salmonella typhi*
6 mg/µl	9.76± 1.65	15.33± 1.12	7.93± 0.66	10.23± 0.96	13.12± 1.03
12 mg/µl	14.50± 1.25	13.57± 0.87	12.83± 0.95	14.27± 1.22	16.06± 0.97
18 mg/µl	6.32± 1.00	12.13± 0.78	11.13±1.65	13.16±0.57	10.062±1.32

Table10:-Antifungal activity of methanolic extracts of *Berberis lycium* against selected fungal strains

Extracts concentration	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeroginosa*	*Escherchia coli*	*Salmonella typhi*
6 mg/µl	6.89± 0.11	16.20± 1.89	13.44± 0.53	10.67± 1.30	11.65± 1.75
12 mg/µl	10.33± 0.88	14.12± 0.192	17.00± 0.48	16.27± 0.93	16.11± 0.82

18 mg/µl	10.80± 0.58	9.89± 0.63	13.62± 0.87	9.17± 1.01	14.77± 1.13

Discussion:-

In the present research work the phytochemical investigation of methanolic, ethanolic and chloroform extracts of*Berberis lycium* and Pharmacological activities of methanolic extracts (Anti-inflammatory and analgesic activity) and antibacterial activities in methanolic, ethanolic and chloroform extracts was carried out. Qualitative analysis of *Berberis lycium* was carried out for the detection ofalkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides and proteins. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins, glycosides and protein were found absent in the aqueous extracts. Flavonoids, carbohydrates, saponins, phenols and terpenoids were found present in in the rhizome methanolic and ethanolic extracts. Highest amount of flavonoids was found in the chloroform extract as (14.20 ± 0.15mg/ml) followed by Alkaloids (12.10 ± 0.15mg/ml), phenolics (10.45 ± 0.10mg/ml), Saponins (06.22 ± 0.14mg/ml) and lowest amount of Tannins was found in (04.60 ± 0.65 mg/ml). The flavonoids was found in highest amount in methanolic as (17.55 ± 0.10mg/ml), followed by phenols (13.25 ± 0.50mg/ml), Tannins (11.55 ± 0.30mg/ml), Alkaloids (10.05 ± 0.10mg/ml) and Saponins was found in lowest amount (08.40 ± 0.45 mg/ml). At the doses of 200,400,600 mg/kg b.w the administration of extract produced a significant anti-inflammatory activity at 3 hours with paw oedema inhibition of 28%, 41 % and 59 % respectively, while the standard drug aspirin inhibited paw oedema of 68%.% inhibition of pain responses in extract 200 mg/Kg 17%, in extract 400 mg/Kg is 31% and in extract 600mg/Kg is 61%. The percentage inhibition of pain by 150mg/Kg aspirin was found to be 78% as compared to the other doses of the *Berberis lycium*.Extract of *Berberis lycium* were active against all bacterial species and showed different range of zone of inhibition. The most active among the extracts was with (17.00± 0.48 mm) zone of inhibition at the concentration of18 mg/µl against *Pseudomonas aeroginosa*. Fallowed by *Escherchia coli*(16.27±0.93mm), *Shigella flexneri* (16.20± 1.89mm) and *Salmonella typhi* (16.11± 0.82) with concentration of 12 mg/µl. And the ethanolic extracts showed maximum zone of inhibition (16.06± 0.97 mm) at the concentration 12 mg/µl against *Salmonella typhi*fallowed by *Shigella flexneri*(15.33± 1.12 mm) at the concentration of 6 mg/µl and lowest amount of inhibition was showed against *Pseudomonas aeroginosa*(11.13±1.65mm) and *Salmonella typhi* (10.062±1.32mm). The chloroform extracts showed maximum inhibition against *Pseudomonas aeroginosa*(16.63± 0.65mm) at the concentration of12 mg/µl, followed by *Salmonella typhi*(16.18± 1.43mm) at the concentration of 6 mg/µl,*Staphylococcus aureus*(*14.40± 1.34*) at the concentration of 12 mg/µl.Phytochemical ingredients which are present in plant samples are known to be biologically active compounds and they are responsible for diverse activities such as antioxidant, antimicrobial, anticancer, antifungal, and antidiabetic (Hossain & Nagooru, 2011).Wide variety of pharmacological activities showed by different phytochemicals, which may help in protection against chronic diseases. Tannins, flavonoids, saponins, glycosides, and amino acids have anti-inflammatory and hypoglycemic activities. Steroids and terpenoids shows central nervous system (CNS) activities and analgesic properties. Because of their antimicrobial activity saponins are involved in plant defense system (Ayoola *et al*., 2008). These phytochemicals showed antimicrobial activity through different mechanisms. With proline-rich protein tannins have been found to form irreversible complexes (Shimada, 2006) resulting in the inhibition of cell protein synthesis. (Parekh and Chanda, 2007) reported that tannins are known to react with proteins to deliver the typical tanning effect which is essential for the treatment of ulcerated or inflamed tissues. Herbs that have tannins as their key components are astringent in nature and are used for treating intestinal disorders such as dysentery and diarrhea (Dharmananda, 2003).Tannins and their derivatives are phenolic compounds considered to be primary antioxidants or free radical scavengers (Barile *et al*., 2007). These observations therefore support the use of *Berberis lycium* in herbal cure remedies, thus suggesting that *Berberis lycium* has potential as a source of important bioactive molecules for the treatment and prevention of cancer. The presence of tannins in *Berberis lycium* supports the traditional medicinal use of this plant in the treatment of different ailments. Alkaloid was another phytochemicals constituent's observed in the extract of *Pteris quadriaurita*. One of the best common biological properties of alkaloids is their toxicity against cells of foreign organisms. These activities have been widely studied for their potential use in the reduction and elimination of human cancer cell lines (Nobori, *et al*., 1994). One of the largest groups of phytochemicals as alkaloids in plants which have amazing effects on humans and this has led to the development of powerful pain killer medications (Kam and Liew, 2002). (Just *et al*., 1998) shown the inhibitory effect of saponins on inflamed cells. Saponin was found to be present in *Berberis lycium* extracts and has supported the usefulness of this plant in managing inflammation. Flavonoids, another phytochemicals shows a varied range of pharmacological activities like anti-inflammatory, antimicrobial, analgesic, anti-angionic, cytostatic, antioxidant and anti-allergic properties (Hodek *et al*., 2002).Several reports are presented on flavonoid groups which showing high potential biological activities such as anti-inflammatory, antioxidant, antiallergic reactions (Thitilertdecha *et al*., 2008). In the

crude extractsthe bioactive compounds such as flavonoids and tanninsphytochemicals were present. Yet, these phytochemicals compounds were inducing the antimicrobial and antioxidants activities. By fractionationthe amount of active constituents in the crude extractsmight be dilute or improved their concentrations (Anyasor *et al*., 2010).

Conclusion:-

The above results confirmed that *Berberis lycium* has better anti-inflammatory analgesic and antibacterial activity. The pharmacological activity of the *Berberis lycium* may be due to the presence of phytochemical constituents. Some of these compounds possess analgesic, anti-inflammatory, antipyretic and antifungal activities. Further studies involving the purification of the chemical constituents of the plant and investigation in the biochemical pathway may results in the development of a potent analgesic, anti-inflammatory, anti-pyretic and antifungal agent with low toxicity and better therapeutic index.

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