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Preliminary phytochemical, essential element analysis and antimicrobial activities of ethanolic extract of *Lotus Corniculatus*

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Abstract

Leaves of family fabaceae Lotus *corniculatus* were collected for preliminary phytochemical, antimicrobial and essential element analysis. The antimicrobial activities of ethanolic extract of *lotus corniculatus* were investigated against *Escherichia coli*, *Pseudomonas aeruginosa, Salmonella typhi*, *Klebsiella pneumoniae* and some fungal strains i.e. *aspergillus Niger* and *aspergillus Flavus* were susceptible to ethanolic extracts of this plant .The present study deals with the development of novel plants sources having antimicrobial activities. In this respect, ethanolic extracts of various parts of this plant were investigated against some bacterial and fungal strains. Various phytochemicals like flavonoids, tannins, alkaloids, glycosides, phlobotannins, and cardiac glycosides were analyzed qualitatively and quantitatively. Similarly some essential elements were analyzed by using atomic absorption spectrometer. Moreover, the current research work will help to introduce new and cost effective antibiotic resources and will help to solve the problem of resistance develop by microbes against allopathic antibiotics.

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Introduction

Plants are uses as remedies for the treatment of diseases since prehistoric period. Plants are important source for maintaining health and especially in the last few decades extensive study has been carried out for developing natural therapies. Traditional medicines are used in all parts of the world because they are economical than modern medicines (Jehan *et al.,* 2011). Medicinal plants are safe natural resources that have been tested for hypoglycemic, antimicrobial and biological activities. They also play important role in modern medicines (Hassawi *et al.*, 2006; Bhatt *et al.*, 2009). It is obvious that most synthetic drugs have their origin from plants (Ríos *et al.*, 2005).

In developing countries including Pakistan, diseases that are caused by microorganism are becoming a challenge for health and economy therefore, control measures are necessary. Microorganisms caused a multiple drug resistance due the improper use of commercial antibiotics resulting in increased risk of toxity, immune suppression, hypersensitivity and allergic reactions. In such a situation, the scientists should develop new antimicrobial substances (Anely *et al.*, 2007) **.**

Phytochemicals are divided in two main groups (Krishna *et al.*, 2009) amnely which include proteins, sugars, amino acids and chlorophyll etc. and secondary one consists Saponins, flavonoids, tannins, terpenoids, alkaloids, essential oils and phenolic compounds etc. ((Krishnaiah *et al.*, 2007; Edeoga *et al.*, 2005). Most of the phytochemicals have shown valuable therapeutic activities such as insecticidal (Kambu *et al.*, 1982). Antifungal, antibacterial, anticonstipative, spasmolytic, antiplasmodial and antioxidants activities etc.((Kambu *et al.*,1982; Lemos *et al.*, 1990; Ferdous *et al.*, 1992; Sontos *et al.*, 1998; Benoitvical *et al.*, 2001; Vardar-unlu *et al.*, 2003). Terpenoids exhibit various important pharmacological activities i.e., anti-inflammatory, anti-cancer, anti-malarial, inhibition of cholesterol synthesis, ant-viral and anti-bacterial activities. Terpenoids are very important in attracting useful mites and consume the herbivorous insects. (Kappers *et al.*, 2005). Alkaloids are used as anaesthetic agents and are found in medicinal plants. (Hérouart *et al.*, 1998).

Infectious diseases are the leading causes of death throughout the world that accounts for nearly one half of all death in the tropical countries, which are also becoming a serious problem in developed countries. It is calculated that infectious diseases are the main causes of death in 8% of the 9 deaths occurring in United States (Demissew and Dagne *et al.*, 2001). In addition, antibiotics are sometime associated with adverse effects including hypersensitivity, immuno suppressant and allergic reactions. Given the alarming incidence of antibiotic resistance in bacteria of medical importance, there is a constant need for new and effective therapeutic agents. Therefore, there is a need to develop alternative antimicrobial drugs for the treatment of infectious diseases from medicinal plants. *Lotus corniculatus* is medicinally very important plants and use extensively in herbal pharmaceutical formulations and also used by local medical practitioners for a variety of human diseases. Hence the aim of this study was to determine the phytochemical constituents and to investigate the antimicrobial properties so as to ascertain their uses in traditional medicines. After a thorough study it was found that no such study was performed on locally grown plant *Lotus corniculatus* and due to its wide range medicinal application by local practitioner it was necessary to find out the broad range importance of the mentioned plant and to disclose its further medicinal applications to local markets and pharmaceutical industries as well.

Therefore the aim of current piece of research work is to perform qualitative and quantitative investigation of phytonutrients of locally collected plants *Lotus corniculatus*. More ever this work was further proceeded to find out the antimicrobial activities of ethanolic extracts of this plant against some gram positive and gram negative bacterial as well some fungal strains. (Mahato *et al.*, 1997). Similarly

essential elemental analysis was carried by applying atomic absorption spectrometric technique.

Material and methods

Crude extracts preparation

About 50g of the ground leaves of *Lotus corniculatus* were soaked in ethyl alcohol for two days and shaken it occasionally. The extracts were then filtered using Whattman filter paper.

Preliminary qualitative phytochemical analysis

For qualitative screening of ethanolic extracts of the leaves different standard procedure were used.

Qualitative test for flavonoids

A part of powdered plant sample was warmed along with 10ml of solvent ethyl acetate by using steam bath for three mints. It was further filtered and 4ml of filtrate was shaken with 1ml of dil Ammonia solution followed by addition of 1 ml concentrated H_2SO_4. The appearance of yellow coloration indicates the presence flavonoids in the sample.

Qualitative test for reducing sugars

Benedict's test

To 2.5ml of Benedict's solution add test solution in a test tube. Warm it over water bath for about 5 mints. Solution shows green, red or yellow coloration indicates the positive sign of reducing sugar in sample solution.

Qualitative test for tannins

Lead acetate test

3ml of extract was added with 3ml of lead acetate solution in a test tube. Formation of white precipitate indicates the presence of tannins.

Qualitative test for terpenoids

5ml of plant extract was added with 2ml of $CHCl_3$ and carefully with 3ml of Conc H_2SO_4 to form a layer. Appearance of reddish brown coloration gives a positive indication for the presence of terpenoids.

Qualitative test for alkaloids

2-3ml of plants sample extract was added with 2% H_2SO_4 in a test tube and further warmed for two minutes. The reaction mixture was filtered through Whattman filter paper and was added with few drops and few drops of Dragondroff's reagents. The appearance of orange red ppt shows the presence alkaloids in the plants sample extracts. (Tyler and Herbalgram *et al.*, 1994; Harborne *et al.*, 1973).

Qualitative test for phlobotannins

Plant leaves powder sample was shaken with distilled water in a test tube, and filtered to take plant extract. Then to plant extract, 1% aqueous hydrochloric acid was added and plant sample was then boiled with the help of Hot plate stirrer. Formation of red colored precipitate indicates the presence of phlobotannins.

Qualitative test for steroids (Liebermann- Buchards test)

Plant extract was added with chloroform and filtered. Acetic anhydrides was added to the filtrates, further boiled and cooled. Concentrated Sulphuric acid was added to the test tubes .The formation of brown ring at the junction give a positive indication for the presence of steroids.

Qualitative test for saponin

10ml of filtrate was mixed with 5ml distilled water by shaking. Formation of creamy mass of small bubbles shows us the presence of saponin.

10 ml of distilled water was added to10 ml of the test solution and shaked well and observed for small bubbles or frothing (Tyler and Herbalgram *et al.*, 1994; Harborne *et al.*, 1973).

Qualitative test for cardiac glycosides

5ml of sample extract was mixed with two ml of glacial acetic acid containing 1 drop of $FeCl_3$ solution. This was further added with 1ml of Conc. H_2SO_4. A brown ring of interface indicates a deoxysugar characteristic of cardiac glycosides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just gradually throughout thin layer.

Qualitative test for anthraquinone

Half gm. of the sample extract was added with 10ml of sulphuric acid and further boiled and filtered while hot. The filtrate was also shaken with 5ml of chloroform. The chloroform layer was transferred to another test tube and was added with one ml of dilute ammonia solution. The resulting solution was observed for color changes which indicate the presence of anthraquinone.The results shown in Table 1.

Qualitative test for anthraquinone glycosides

Modified borntragers test

To 5ml of sample extract add 5% of $FeCl_3$ 5ml of dil.HCl and warm it for a min over boiling water bath ,cool and add benzene, shake well, separate the organic layer ,add equal volume of ammonia, pink or red color indicates presence of anthraquinone glycosides.

Qualitative test for carbohydrates

Molish'test

2-3ml of ethanolic extract was added with alpha nepthol solution, shake well and add Conc. H_2SO_4 from sides of test tube. Violet ring formation at the junction of two liquids indicates presence of carbohydrates.

Qualitative test for protein

Million's test

Mix 3 ml with 5ml million's reagent, then white ppt formed, warms it then ppt will turn brick red or ppt dissolves given red color solution.

Quantitative analysis of phytochemicals

Alkaloid determination using Harborne (1973) method

5.0 g of the plants powdered sample was taken into a beaker and was added with 200 ml of 10% acetic acid in ethanol and covered and allowed to keep for 4 h. This mixture was further filtered and the extract was concentrated on a boiling water bath to one-quarter of the solution original volume. It was further added with drop wise Conc NH_4OH to the extract till the completion of the precipitate formation. The whole solution was allowed to settle down and the precipitate was collected and washed with dilute NH_4OH and then filtered. The residue is the alkaloid, which was dried and weighed. (Harborne *et al.*, 1973)

Saponin determination

Obadoni and Ochuko *et al.*, 2001 applied the same method for saponin determination. 20gm of the plant samples were ground and put into conical flask and further mixed with 100ml of 20% aqueous ethanol solution. The sample taken was warmed over water hot bath for four hours with regular stirring at 55°C. The mixture was filtered and the residues were re-extracted again by adding 20% of 200cm^3 ethanol. The combined extracts were evaporated to 40 ml over water bath at about 90°C. The concentrate was further allowed to transfer to a 250 ml reparatory funnel and further added with 20 ml of diethyl ether and shaken vigorously.

The aqueous layer was collected and stored while the ether layer was discarded, proceeded through a repeated purification process. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 5% of 10 ml of brine solution. The rest of solution was warmed over boiling water bath. The samples were dried in oven after evaporation to a constant value; percentage weight of saponin content was calculated.

Flavonoid determination by of Bohm and Kocipai-Abyazan (1994) method

10 g of the plant sample was added repeatedly with 100 ml of 80% methanol solution in water at room temperature. The whole mixture was filtered through Whattman filter paper No 42 (125 mm). The filtrate was later transferred into a crucible and evaporated into dryness over a heating water bath and calculated to a constant percent weight.

(Williamson and Manach *et al.*, 2005; Mattila and Hellström *et al.*, 2007).

Determination of antimicrobial activities

Culture media used

Nutrient agar media was used for culturing and growth of microorganisms used in current study. Nutrient broth was used for inoculation, shaking incubation and standardization of microorganisms under consideration in this study. The composition of different media used for culturing and standardization of this study is given in the following tables.

Preparation of media

The required amount (2.8 gl^{-1}) of nutrient agar and (1.3 gl^{-1}) nutrient broth medium were prepared in distilled water and poured into conical flasks (20ml/flask). About 7-8 ml/test tube of the nutrient broth was poured into the test tubes. All the media, flasks and test tubes were plugged with cotton wool and sterilized in an autoclave at 1.5 pounds pressure and 121°C Temperature for 15 minutes. The nutrient agar media after sterilization was poured in aseptic environment into sterilized Petri plates in laminar flow hood. In order to avoid contamination sterile environment was maintain during pouring of the agar medium. The media was allowed to solidify for about an hour, after solidification these Petri plates were placed in an incubator at 37 °C for twenty-four hours in inverted position to avoid the water loss from the medium. After 24 hours, the contaminated plates were discarded and the un-contaminated plates were used for culturing bacteria and fungi. The nutrient broths in the test tubes were used for standardizing microbial culture while nutrient broths in the flasks were used for shaking incubation of microorganisms.

Microbial strains used

Two gram-negative and one gram-positive bacterial strain and two fungal strains were selected for the current study.

Preparation of stock solutions and standards

Ethanol extracts of leaves of *Lotus corniculatus* were evaluated for their antimicrobial activities. The crude extracts of the leaves were diluted and adjusted to 5µg/ disc in DMSO (dimethyl sulfoxide) solvent.Morever Nilidixic acid 30µg/disc was used as standard or positive control against positive and negative bacterial strains while Fluconazole 25µg/disc was utilized against fungus strains.

McFarland turbidity standard

This standard solution was prepared by adding 0.5 ml of 1.175% w/v $BaCl_2.2H_2O$ solution to 99.5 ml of 1% v/v sulfuric acid solution. McFarland solution was poured into test tube and sealed with paraffin. It could be used up to 6 months and then discarded. It is stored in dark at room temperature. The fine white precipitate of barium chloride is mixed by shaking will before each use. The accuracy of the density of the standard McFarland solution is checked by spectrophotometer .The absorbance at 625 nm should be 0.08 to 0.1. The standard McFarland solution gives count of 10^8 colony forming unit per ml.

Inoculation of culture

The original cultures of microbes were refreshed by inoculating it into the solidified freshly prepared media in the Petri plates. The refreshed cultures of microbes were inoculated into nutrient broth and incubated in shaking incubator for the uniform growth.

Disc diffusion susceptibility method

The following standard procedure was used for the determination of antimicrobial potential as described by Aida *et al.*, 2001.

The bacterial cultures were adjusted to 0.5 McFarland turbidity standards and inoculated into nutrient agar in Petri plates. The Aspergillus niger and Aspergillus flavus were adjusted to 10^8 cfu/ml.6 mm Whatmann-1 filter paper disc were put on each plate and extracts were applied in concentration of 1mg and 2mg/6µl and 12µ volumes previously seeded with bacteria and fungi standardized with 0.5McFarlandturbidity standard. Bacterial and fungal cultures were then incubated at 37°C for 24 hours.

Result and discussion

This study revealed the presence of numerous phytonutrients as very important and active medicinal constituents of plants. The roots of *lotus*

corniculatus show the presence of important phytochemicals such as alkaloids, steroids, Saponins, tannins, reducing sugars, anthraquinone, anthraquinone glycosides, carbohydrates and proteins which give positive results while some other i.e. terpenoids, cardiac glycosides and phlobotannins give negative results. The stem show the presence of alkaloids, tannins, saponin, flavonoids, steroids, and reducing sugars and anthraquinone, terpenoids, phlobotannins and glycosides were found absent. The leaves show the presence of alkaloids, tannins, saponin, steroids and reducing sugars while anthraquinone, terpenoids, flavonoids, phlobotannins and glycosides were found absent. The above discussion revealed that roots of *lotus corniculatus* are more rich with phytochemicals while leaves and stems also contains many medicinally important phytochemicals in it. The following results were obtained by disc diffusion method for the plant *Lotus corniculatus***.** The determination of percentage extractive value is an important parameter for standardization and quality assessment of medicinal plants, because it shows the quantity of the active phytochemicals of the plant. The quantity of various constituents of the same plant varies in different solvents. The polarity and nature of phytochemical of different plants are also different from each other. The same plant grown in different areas also differ in phytochemicals because of different climatic conditions. In current study percentage, extractive value of root, stem and flowers of *lotus corniculatus* were determined in ethanol and the results are shown in table 1.

Table 1. Qualitative screening results of leaves of *Lotus corniculatus*.

S.No	Phytochemical constituents	Ethanolic extract of leaves
1	Alkaloids	+ve
2	Flavonoids	+ve
3	Steroids	+ive
4	Saponins	+ve
5	Tannins	+ve
6	Reducing Sugar	+ve
7	Terpenoids	_ve
8	Anthraquinone	+ve
9	Anthraquinone Glycosides	+ve
10	Cardiac Glycosides	-ive
11	Phlobotannins	-ve
12	Carbohydrates	+ve
13	Protein	+ve

Table 2. Microorganisms used in the present study.

S.No	Specie	Type
1	Escherichia col	Gram-negative
2	Pseudomonas aeruginosa	Gram-negative
3	Staphylococcus aureus	Gram-positive
4	Salmonella typhi	Gram-negative
5	K. pneumoniae	Gram-negative
4	Aspergillus Niger	Fungus
5	Aspergillus Flavus	Fungus

The results of antibacterial activity of the decoction of *lotus corniculatus* of paper disc and agar well diffusion methods are depicted in table 3.The activity against Gram Positive tested pathogens was very prominent, similar to standard drug Nilidixic acid, While Pseudomonas aeruginosa of the Gram Negative

nature pathogens was not susceptible but the rest of them i.e. *Escherichia coli*, *Salmonella typhi* and *K. pneumoniae* are very susceptible same like the standard drug used. Notably, both the methods were equally showing nice response against tested pathogens. As shown in table 3.

Table 3. Antibacterial and antifungal activity of decoction of *Lotus corniculatus* in paper disc method. Diameter of Zone of inhibition (mm).

S. No	Bacterial and fungus strains for test	Ethanol Sample extract - 50µg/disc	Standard Nilidixic acid Fluconazole 30µg/disc and 25µg/disc
1	Escherichia col	00	13±0.05
2	Staphylococcus aureus	08±0.2	12±0.2
3	Pseudomonas aeruginosa	00±0.0	13±0.01
4	Salmonella typhi	11.0±0.01	12±0.02
5	Klebsiella pneumoniae	13.5±0.02	15.5±0.02
6	Aspergillus Niger	10±0.1	15±0.02
7	Aspergillus Flavus	12±0.3	14±0.05

Table 4. Percentage of essential elements in *Lotus corniculatus*.

Sample ID	Cu	Pb	Ni	Cr	Zn	Fe	Mn	Na	K	Ca
Mg/1g or %age	3.8	5.8	1.6	5.8	54.8	69.6	45.4	0.3%	0.85%	1.75%

The ethanolic extract of *lotus corniculatus* shows interesting sensitivity against the fungal strains i.e. *aspergillus Niger* and *aspergillus Flavus* similar to the standard drug of Fluconazole can be seen in figure 3.

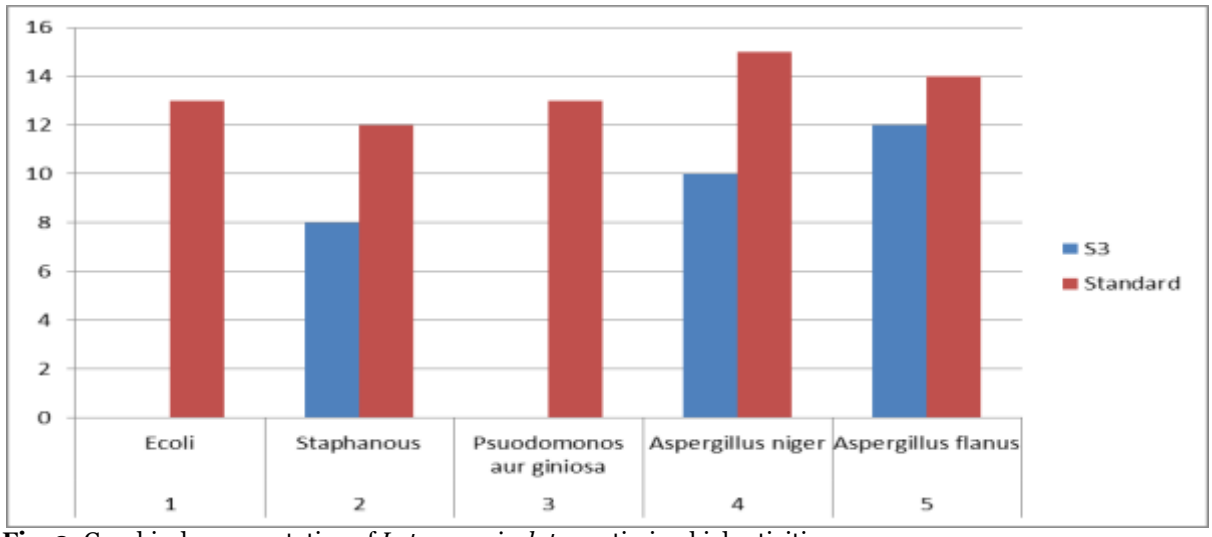

Fig. 3. Graphical representation of *Lotus corniculatus* antimicrobial activities.

In the present study the local medicinally used plant *lotus corniculatus* extract shows interesting activity against the gram positive pathogen of *Staphylococcus aureus* which is the major cause of the infection of upper respiratory tract. For this reason our study would help in copping the upper respiratory tract problems and more solely solved more complicated problems by isolating individual components of the plants extract.Similraly it also shows more effectiveness against gram negative pathogen i.e. *Escherichia coli*, *Salmonella typhi* and *K. pneumoniae* which are the major cause of stomach problems, intestinal as well as lungs inflamations.so the current results of the plant tested will be helpful in future by designing more medical formulations against such infectious diseases.

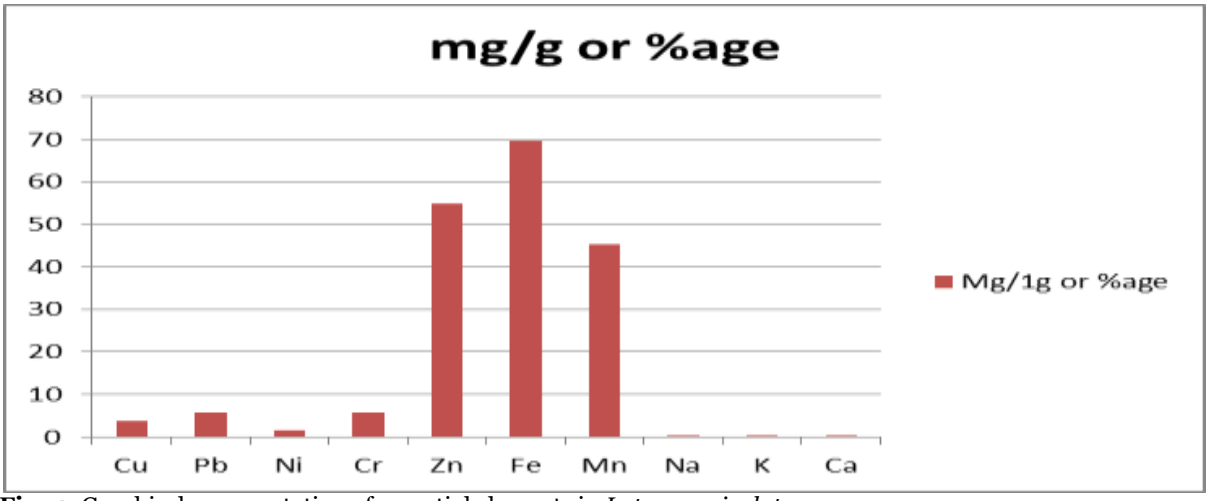

Fig. 4. Graphical representation of essential elements in *Lotus corniculatus*.

Essential element analysis of the leaves of plant shows that it contains many important essential elements. This analysis was preformed through atomic absorption spectrophotometer for the quantification of Cu, Pb, Ni, Cr, Zn, Fe, Mn, Na, K, Ca elements in the leaves of *Lotus corniculatus*. The results shown in table 4 revealed that Mn was present in high concentration of 248.8mg/kg as compared to other elements. The concentration of Fe, Zn, Cu, Ni, Pb, Cr were in the moderate ranges of 150, 48.2, 9.4, 6.2, 5.4, 4.8mg/kg respectively while the concentration of Ca, K and Na were 1.3, 0.25 and 0.2% respectively.

Conclusion

The selected plant is rich source of secondary metabolites i.e. alkaloids, steroids, Saponins, tannins , reducing sugars, anthraquinone, anthraquinone glycosides, carbohydrates, terpenoids, cardiac glycosides and proteins. Medicinal plants play a major role in copping various diseases. The anti-inflammatory, anti-bacterial. Anti-fungal, anti-viral and anti-malarial activities of plants are due to the presence of the mentioned secondary metabolites, therefore the ethanolic extract of *Lotus corniculatus* shows amazing activities against some gram-negative bacterial strains i.e. *Escherichia coli*, *Salmonella typhi* and *K. pneumoniae* which are the major cause of stomach problems, intestinal as well as lungs inflammations and one gram-positive strains of *Staphylococcus aureus* which is the major cause of the infection of upper respiratory tract. Moreover it also shows more susceptibility against two fungal strains. Similarly some essential elements were analyzed by using atomic absorption spectrometry which is very essential for human health. Thus the phytochemical and antimicrobial analysis of medicinal plants are very crucial and have commercial interest in both research institutes and pharmaceutical companies for the synthesis of new drugs for the treatment of many diaseses.Thus we hope that important phytochemical and antimicrobial properties identifies of the Lotus *corniculatus* plants obtained from district Peshawar will help us in copping various diseases of our region

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References

Ashok K, Sathish Kumar M, Narendra Babu A, Eswar tony D, Rama Rao NV, Divya Sri G, Dastagiri M. 2014. Pharmacological investigation of leaf extract of Typha angustata for depressant activity. Asian Journal Pharmacology and Toxicology **2(3),** 1-03.

Anely MN, Dogan Y, Gauarrera P. 2007. Plants traditionally used to make brooms in several

European countries. Journal of Ethnobiology and Ethnomedicine **3(20),** 1946 - 4269. http://dx.doi.org/10.1186/1746-4269-3-20

Bhat S, Mercy L, Chethan K, Sukesh KV, Chandrasheker KR. 2009. Anti-microbial spectrum and Phytochemical study of Hopea parviflora Biddom saw dust extracts. Journal of Phytology **1(6),** 469-474.

Benoitvical F, Valentin A, Mallic M, bassierc JM. 2001. Anti-plasmodialactivity of *Colchlospermum planchonii* and *C. tentorium* tubercle essential oils. Journal of Essential Oil Research **13(1),** 65-67. http://dx.doi.org/10.1080/10412905.2001.9699609

Demissew S, Dagne E. 2001. Basic and Applied Research on Medicinal Plants of Ethiopia, In: Proceedings of National Workshop on Conservation and Sustainable Use of Medicinal Plants in Ethiopia, Addis Ababa, p. 29.

Edeoga HO, Okwu D, Mbaebie BO. 2005. Phytochemical constituents of some Nigerian Medicinal plants. African Journal of Biotechnology **4(7),** 685-688. http://dx.doi.org/10.5897/AJB2005.000-312.

Ferdous AJ, Islam SM, Ahsan M, Hassan CM, Ahmad ZV. 1992. *In vitro* antibacterial activity of the volatile oil of *Nigella sativa* seeds against multiple drug-resistant isolates of *Shigella* spp and isolates of *Vibriocholerae* and *Escherichia coli:* Phytotherapy Research **6(3),** 137-140. http://dx.doi.org/10.1002/ptr.2650060307.

Harborne JB. 1973. Phytochemical methods, London Chapman and Hall, Ltd, 49-88 p.

Hassawi D, Abeer K. 2006. Antimicrobial activity of medicinal plants against Candida albicans. Journal of Biological Sciences **6(1),** 109-114.

Hérouart D, Sangwan RS, Fliniaux MA, Sangwan-Norreel BS. 1988. Variations in the Leaf Alkaloid Content of Androgenic Diploid Plants of Datura anoxia. Planta Med. **54,** 14-17. http://dx.doi.org/10.1055/s-2006-962320

Jehan B, Huma A, Mubarak AK, Ayyub K, Muhammad S, Mohammad S, Amjad I and Mohammad T. 2011. Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion method. African Journal of Biotechnology **10(85),** 19825. http://dx.doi.org/10.5897/AJB11.229.

Kambu K, midi Phenzu, N Coune C, Wauter JN, Angenot L. 1982. Plants Medicine ET Phytotherapie, p. 34.

Kappers IF, Aharoni A, van Herpen TW, Luckerhoff LL, and Dicke M. 2005. Genetic engineering of terpenoids metabolism attracts bodyguards to Arabidopsis. Science **309,** 2070-2072. http://dx.doi.org/10.1126/science.1116232

Krishnaiah D, Sarbatly R, Bono A. 2007. Phytochemical antioxidants for health and medicine – A move towards nature. Biotechnology Molecular Biology Review **2(4),** 097-104.

Krishnaiah D, Devi T, Bono A, Sarbatly R. 2009. Studies on Phytochemical Constituents of Six Malaysian Medicinal Plants. Journal of Medicinal Plants Research **3,** 67.

Lemos TLG, Matos FJA, Alencar JW, Crareiro AA, Clark AM, Chesnary JD. 1990. Antimicrobial activity of essential oils of Brazilian plants. Phytotherapy Research **4(2),** 82-84. http://dx.doi.org/10.1002/ptr.2650040210

Mattila P, Hellström J. 2007. Phenolic acids in potatoes, vegetables, and some of their products. J. Food Compos Anal. **20,** 152–60. http://dx.doi.org/10.1016/j.jfca.2006.05.007.

Mahato SB, Sen S. 1997. Advances in terpenoids

research, 1990-1994. Phytochemistry **44(7),** 1185-1236. http://dx.doi.org/10.1016/S0031-9422(96)00639-5

Ríos JL, Recio M C. 1982. Medicinal plants and antimicrobial activity. Journal of Ethanopharmacology **100 (1-2)**, 80-84. http://dx.doi.org/10.1016/j.jep.2005.04.025

Santos FA, Rao VSN, Silveria ER. 1998. Investigations on the antinociceptive effect of *Psidium guajava* leaf essential oil and its major constituents. Phytotherapy Research **12(1),** 24-27. http://dx.doi.org/10.1002/(SICI)1099-1573(19980201)12:1<24:AID-PTR181>3.0.CO;2-B.

Tyler V. 1994. Phytomedicines in Western Europe: their potential impact on herbal medicine in the United States. Herbalgram **30,** 24-30.

Vardar-unlu G, Cadan F, Sokmen Deferera A, Polissiou M, Sokmen M, Donmez Tap Bektas E. 2003. Antimicrobial and Antioxidant Activity of the Essential Oil and Methanol Extracts of *Thymus pectinatus* Fisch et Mey. Var. *pectinatus* (Lamiaceae) Journal of Agriculture Food Chemistry **51(1),** 63-67. http://dx.doi.org/10.1021/jf025753e.

Williamson G, Manach C. 2005. Bioavailability and bio efficacy of polyphenols in humans. II. Review of 93 intervention studies. American Journal of Clinical Nutrition **81(Suppl 1),** S 243–55.