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Biochemical screening and antibacterial activates of selected medicinal plants from District Harnai, Balochistan Pakistan

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ABSTRACT
This study aimed to carry out the phytochemical and antibacterial activities of the selected medicinal plants from Harnai Balochistan Pakistan. The qualitative and quantitive phytochemical investigation was carried out in four different solvents ethanol, methanol, acetone and chloroform for selected medicinal plant (Olea ferruginea, Tulipa lehmanniana, Pistacia khinjuk and Bunium persicum) and the antimicrobial activity against multi drug resistant strains and environmentally collected strains were carried out. The qualitative phytochemicals finding shows the presence of flavonoids, sugar, protein, phenolic compounds, terpenoids, tannins, cardiac glycerides, steroids, alkaloids, amino acids uniformly in all plants species in all four solvent extracts, glycosides are present in all four species with acetone solvent extracts and is absent in Tulipa lehmanniana, Pistacia khinjuk and Olea ferruginea with chloroform solvent extract and also with ethanol extract in Tulipa lehmanniana and Pistacia khinjuk and it is also absent in Pistacia khinjuk with methanol extracts. Anthroquinone is absent in Tulipa lehmanniana, Pistacia khinjuk and Bunium persicum with chloroform extract but are present in the rest of all extract solvent in all species, fixed oil and saponins is only absent in Tulipa lehmanniana with methanol extract and in Olea ferruginea with ethanol extract solution while it is present with all the rest of solution extract in all sour selected plant species.

The quantitative biochemical investigation revealed that the contents of carbohydrates was reported in Bunium persicum 2.83 µg/mg in the methanol solution, the highest Phenolic contents is quantified in Tulipa lehmanniana in the chloroform

extract i.e. 16.25 µg/mg extracts, Tulipa lehmanniana have the highest percentage of alkaloids in the methanol extracts (41.2%) Olea ferruginea have the highest percentage of saponins in the ethanol extracts (15.2%) and all the extracts. Bunium persicum have the highest percentage of Proteins in the ethanol extract (10.4 µg/ml) followed by Tulipa lehmanniana with (7.2 µg/ml) in the ethanol extracts Solution. The antibacterial activity of the chloroform extracts of Tulipa lehmanniana shows a very good activity against Staphylococcus aureus (MDR) and Escherichia coli bacteria, the acetone extracts of Tulipa lehmanniana has also shown very good activity against Bacillus brevis bacteria. The ethanolic, methanolic, acetone and chloroform extracts of Pistacia khinjuk have showed moderate activity against Staphylococcus aureus (MDR). It is concluded that the medicinal plants wealth of the area should be further investigated for clinical and pharmaceutical potential in order to natural drug discovery development at regional and global level

Keywords: *Photochemistry, Antibacterial activity, Multi drug-resistant strains, Harnai.*

1. INTRODUCTION

Phytochemicals are dealing with the study of plant chemicals known as natural products, and changes occurring in these products with passage of time due to alteration in environmental conditions. These natural compounds are also involved in allelopathy, interaction between plants; these depend upon the variation in chemical nature of these compounds produced in particular environmental conditions (Zobel *et al*., 1999).

Phytochemicals are the compounds founds in plants are bioactive that work with nutrients and dietary fiber to shelter against diseases. They are non-nutritive compounds (secondary metabolites) that contribute to flavor and color (Craig, 1999; Agbafor & Nwachukwu, 2011). The organic compounds which are present in medicinal plants have definite physiological action on the human body and these bioactive substances are alkaloids, carbohydrates, flavonoids, steroids, terpenoids and tannins (Edeoga *et al*., 2005).These compounds are synthesized by the primary or secondary metabolism of living organisms. These secondary metabolites are taxonomically and chemically diverse compounds with obscure function. They are commonly used in the human treatment, agriculture, veterinary, scientific research and in many other fields (Goud *et al.,* 2009). A variety of phytochemicals belonging several chemical classes have been shown to have inhibitory effects on all types of microorganisms in vitro (Cowan, 1999). And evidence based plants based drugs can help us to fight against bacterial infections including multidrug resistance (MDR).

According to World Health organization, medicinal plants are the best source to obtain variety of drugs. About 60% of the world population still using traditional medicines, this has compounds obtained from medicinal plants (WHO, 2010). However the properties of such plants should be investigated properly for better understanding of their safety and efficiency (Arunkumar & Muthuselvam, 2009). And 70% of the world population (Developing countries) are still depends upon such traditional medical therapies as their primary source of health care (Khan *et al* 2013). In recent years about 43% of the deaths in the developing countries are due infectious disease. The search of new antimicrobial agent is the need of time due to the resistance of microbes and fatal opportunities of infections (Carballo *et al.,* 2002). The plants based drugs are in practice for thousands of years around the world (Lewis & Elvin-Lewis, 2003).The plant based system are playing an essential role in the health care system and increasing research in the resent past in this fields indicates the positive role plants extracts for health care system (Javed *et al.,* 2012). Most plants have medicinal properties so that their toxicity and effectiveness risk are evaluated (Olagunju *et al*., 2009). The knowledge of plant based chemical constituents would further be valuable and discovering the actual value of folk cure (Mojab *et al*., 2010). Fresh and effective antimicrobial agents with large scale activates from natural sources are need of the day because of the development of resistance of pathogens against antibiotics. American society of microbiology has recommended the development of new antibacterial agents to control the multidrug resistant bacteria pathogens (Jones, 1996).

Plant extracts and phytochemicals with known antimicrobial activity are of great significance in therapeutic treatment. A large number of studies have been conducted throughout the world to make improvement in such efficiency. A large number of plants have been used due to their antimicrobial activity, which is chiefly obtained processing the synthesis of secondary metabolite of plants (Prusti *et al.,* 2008). Multiple drug resistance in plants and human has developed to the unselective use of antimicrobial drugs which are used in the treatment of various infectious diseases. Due to the limited life period of antibiotics has made it necessary to search for new antimicrobial substances from various sources such as medicinal plants. Medicinal plants are the most important areas in search of biologically active new compounds. Medicinal plant and their products are providing unlimited opportunities for new drug leads due to unmatched availability of chemical diversity (Cos *et al*., 2006).Due to this the microbiologist all over the world fell the need of new antimicrobial agents and evaluation of the efficacy of natural plant products as a substitute for antimicrobial agents (Pandian.*et al.,* 2006).

Antibiotic resistance has been increased significantly in the resent years and increasing therapeutic problem for ever, by using the antibiotic resistance inhibitors from plants due to this strategy the chances of resistance to the antibiotics has been overcome (Alagesaboopathi, 2011). Plants are producing verity of chemical compounds to protect themselves against the pathogens in nature. Plant extracts are expected to target specific sites rather than those used by antibiotics which will be active against drug resistant pathogens (Ahmad & Beg, 2001). Medicinal Plants are used for human health since the beginning of humanity in every corner of earth. Now the researcher have diverse their attention towards the safe and sound plants based medicine and biologically active compounds isolated from medicinal plants species used in herbal medicines with acceptable therapeutic index for the development of novel drug (Pavithra *et al.,* 2010). Multiple drug resistance is increasing due to unselective use of antimicrobial drugs in the treatment of various infectious diseases. Mankind is pacing a serious threat due to the antimicrobial resistance acquiring by many pathogenic bacteria which has been an alarming point now a days. Due to the presence of resistant bacteria people will get sick for longer period than ever and some time they will be unable to recover for at all. Due to the concern of side effects of conventional medicine, the use of natural products as a substitute to conventional treatment in healing and treatment of various disease has been increasing with every passing day from last few years (Kumari.*et al*.,2011).

Balochistan is the largest province of Pakistan, poses rich source of herbal medicinal plants, but has not explored systematically for medicinal plants. This province, representing 44% of the total land cover of Pakistan. The climate is arid to semi-arid, ranging from coastal tropical to cool temperate in the north. Major ecological zones are; dry temperate forest, sub-tropical forest, tropical dry mixed deciduous forest, and desert and mangrove forest. This province is blessed with diverse flora and fauna due to diverse ecological conditions (Anonymous, 1998). Balochistan is the home of 5% of the population of Pakistan however a very few studies have been conducted in province regarding ethnobotany but no any phytochemical investigation on the medicinal plants has been carried out yet (Tareen.*et al.,* 2010; Bibi, *et al*., 2014; Bibi.*et al.,* 2015)..Today a large number of medicinal plants at global level are explored for antimicrobial activity and chemical compounds (Evans, 2009). While the medicinal wealth of Balochistan generally and Harnai district particularly yet not explored scientifically particularly to prove the ethnomedicinal claims, none of studies exists on biochemical analysis of medicinal plants of district Harnai. In this regard the present project is conducted to fill the past gaps regarding the biochemical analysis of most useful medicinal plants of district Harnai. Medicinal plants can be use generating income source for indigenous people these valuable resources can be commercially exploited for the benefit of human being and economy of Pakistan, due to its potential for the development as sustainable sources of income to the local people, traders and allied industrial concerns medicinal plants continue to be extensively used as a major source of drugs for the treatment of many health disorders all over the world 80% of the rural population of Pakistan still depends on traditional medicines for their primary healthcare needs (Hocking,1958).

The indigenous people are well acquainted with the properties and uses of plant of their surroundings. Plants have been used as medicines for thousands of years and are used today in their natural as well as processed from. Many medicinal plants which have been forgotten by modern man as a result of his dependence on the quick results of allelopathy medicines and are being rediscovered because of growing awareness of unwanted side effects and other aspects of the later (Alagesaboopathi *et al.,* 2003). Plants have always been the source of medicines and have many uses to mankind. According to some earlier workers (Jain, 1965) medicinal plants have a significant role during pregnancy, birth and postmortem care in many rural areas of the world plants used in women's health related conditions such as female fertility, menorrhea, birth control, pregnancy, birth (parturition), postpartum (puerperium) and lactation, including infant care have been documented for various ethnic groups (Zumsteg & Weckerle, 2007).

Harnai is the district of Balochistan which is enjoying every type of weather, from extreme warm to extreme cold, so that in winter the juniper forest of Zarghoon area are receiving snow fall and the lower areas of district Harnai is shearing its boundary with the warmest district of the province district Sibi, so that is why this area is having diverse medicinal flora from higher mountains to the lower valleys of higher temperature in summer season. The area geographically lies between 67°13'12"-68°24'34"East longitudes and 29°41'59"-30°23'2" North latitudes on the map of the world. The district Harnai is the third smallest district of Balochistan province area wise with an area of 3,075 square kilometers. The total population of the district is 12, 1000 approximately (Anonymous 2011). The local language of tribal people is Tareeno, which is an ancient form of Pashto language which is spoken only in Harnai, in the radius of 21 square kilometers and in some parts of District Ziarat Wanachi area. Urdu, Punjabi, Balochi, Persian Sindhi, Brahavi, is also spoken. Most of the people from district Ziarat and Zarghoon Ghar area are migrate towards lower area of Harnai in winter, and while in summer the people from district Sibi and lower warmer parts of Harnai migrate towards the higher mountain ranges of Harnai along with their live stocks The characteristic features of Harnai are the mountain ranges which are running throughout the district; the highest peak of Khalifat is about 3545 meters above the sea level. The terrain elevation of mountains varies from 192 to 3545 meters above the sea level (Anonymous, 2011). However no study is present on the phytochemical and antibacterial activities of medicinal plants on district flora the present study regarding the use of medicinal plants phytochemical and antibacterial activity will be the first study in the area. Harnai district is chosen for the study site as the area is very important for medicinal plants resources and having the forest of living fossils (*Juniperus excelsa*). A wide range of *Olea ferruginea* forest also exists in the mountain ranges of the area. The most important medicinal plant species of the district including *Achillea wilhelmsii,*

Albizia lebbeck, Allium griffithianum. Allium jacquemontii, Artemisia herba-alba, Artemisia scoparia, .Berberis baluchistanica, Bunium persicum, Calotropis procera. Capparis decidua , Caralluma tuberculata, Citrullus colocynthis,Cleome rupicola ,Cocculus, Ephedra gerardiana, Ephedra intermedia, Ferula baluchistanica, Olea ferruginea, Phyla nodiflora, Pistacia khinjuk, Plantago ciliata, Polygonum barbatum, Rosa beggeriana, Rumex chalepensis, Salvia bucharica, Salvia cabulica, Tamarix karelinii, Tecomella undulate, Teucrium stocksianum, Tribulus terrestris, Triticum aestivum, Tulipa lehmanniana, Typha domingensis, Verbena officinalis, Veronica biloba, Withania coagulans, Xanthium strumarium, Ziziphora clinopodioides, Ziziphora tenuior, Ziziphus jujuba

In Harnai, no proper attention has been given to the investigation of phytochemical and antibacterial activities of medicinal plants in previous studies. The present project will provide a key information for development of polices and proper utilization of the natural resources of wild plants. The present work may provide the new resources of medicinal plants, botanical data based on phytochemical and antibacterial activates for future investigation by Taxonomist, food chemist, pharmaceutical industry. This study is carried conducted in 2016.

2. MATERIALS AND METHODS

2.1 Plant materials Collection and authentication

The present study included four medicinal plant species which are *Bunium persicum, Olea ferruginea, Pistacia khinjuk and Tulipa lehmanniana.* The fresh plant materials were collected from the various areas of District Harnai Balochistan Pakistan. Plant species were further authenticated with the help of plant taxonomists (Dr. Rasol Bakhsh Tareen) Botany Department, University of Balochistan Quetta. (Dr. Mushtaq Ahmad), Department of Plant Sciences, Quaid-i-Azam University Islamabad Pakistan and further identification regarding correct scientific name is conformed from international plants naming index (http://www.ipni.org/). The lists of plants used in this investigation were deposited for herbarium specimen in the University of Balochistan Quetta. The plants included in this investigation are shown in the table No.1 along with their botanical name, family, local name disease treated, voucher no. collection site and plant part used

2.2Extraction of plants extracts

Fresh plant parts *Bunium persicum* (seeds)*, Olea ferruginea* (fruits) *Pistacia khinjuk* (leaves) *and Tulipa lehmanniana* (whole plant) were washed with tape water to remove mud and dusts. The plant parts were to small pieces with the help of a sharp knife. The plants parts were dried under shade until all the water presents in plants were evaporated and the plants material became dried for grinding. The plant materials were pulverized by an electrical grinder and passed through a 40 mesh sieve and fine powder were transferred into air tight containers with proper labeling for future use. 10 grams of each sample were weighted. The powder of each plant (10 gm) were taken in conical flasks of each sample and were dissolved in 50 mL solvent like ethanol, methanol, acetone and chloroform, the mixture were kept for 72 hours on mover and shaker to dissolve the nutrients properly and extracts were collected after every 24 hours for three times in 72 hours. After this the extracts were subjected to filtration and evaporation of each solvent to dryness. The dried extracts of plants were kept in refrigerator for future use in phytochemical and antibacterial activities (Wadood *et al*., 2014).

2.3Chemicals and Glassware

The analytical grade chemicals used in the present phytochemical investigation includes, Nitric acid (HNO3), sulphuric acid (H2SO4), perchloric acid (HClO4), hydrochloric acid (HCl) Potassium sulphate (K2SO4), Sodium hydroxide (NaOH), copper sulphate (CuSO4) sodium sulphate (Na2SO4) sodium carbonate (Na2CO3), Chloroform, acetone, methanol, ethanol, DMSO4, Na2CO3.

Glassware and accessories comprises volumetric flasks, Conical flasks, funnels, beakers, pipits, graduated cylinder, filter paper No.42, petri dishes, gloves, nose masks, paper envelop, shopping bags, plastic bottle, Eppendorf Tubes, micropipettes, racks, stands. The glassware were thoroughly cleaned and dried in an electric oven for about 3 hours prior to use.

2.4 Sample collection and processing

Four medicinal plants were collected from different parts District Harnai Balochistan Pakistan. The medicinal plants were used for the purpose of their phytochemical analysis. The plants were collected and identified using consultancy of Herbarium of Pakistan (Isl), Department of Plant Sciences at Quaid -i- Azam University Islamabad and further conformation for plants were done from flora of Pakistan and international plants naming index (http://www.ipni.org/).

The fresh plant parts/ materials of all four selected medicinal plants were collected from various parts of District Harnai (Bunium persicum, Olea ferruginea, Pistacia khinjuk and Tulipa lehmanniana) Chaperleft, Wamthangi, Zarghoon Ghar area .The plant materials was washed with tape water followed by distal water and then dried at room temperature in shade then placed in paper envelope and dried at 55˚C for 24 hours in electric oven (Wahab *et al*., 2008).

The oven dried sample were ground into fine powder with the help of electric grinder and sieved through 20 mesh sieve, and then the powder were placed in dry and cleaned plastic zip bags and stored at room temperature in desiccators for further phytochemical and antibacterial analysis (Meena *et al*., 2010).

2.5Qualitative phytochemical analysis

The qualitative phytochemical investigations of selected plants extracts were carried out using standard methods of analysis for flavonoids, tannins, carbohydrates, Saponins, cardiac-glycosides, Terpenoids, steroids, Phytosteroids, anthroquinones, phlobatanins, alkaloids, quinines. (Yasuma & Ichikawa, 1953; Gahan, 1984; Wagner, 1993; Evans, 1997; Ramakrishnan.*et al*., 1994).

Test for Flavonoids

For the confirmation of flavonoids in the selected medicinal plants .2mL of each selected plant extract were added to 1mL of 2N sodium hydroxide. The presence of yellow color shows the presence of flavonoids. The method used for detection of Flavonoids in the selected plant in the selected solutions was in accordance Evans, (1997).

(b**) Test for Tannins**

For the purpose of tannins in the selected medicinal plants 1mL of each medicinal plant extract was added to 2mL of 5% ferric chloride. The formation of dark blue or greenish black colors indicates the presence of tannins. The method follow for tannins detection in selected medicinal plants was in accordance Evans (1997).

(c)Test for Carbohydrates

For the confirmation of carbohydrates of the selected medicinal plants in the different solvents extracts. 2mL of each plant extract were added to 1mL of Molish's reagent and few drops of concentrated sulphuric acid. The appearance of reddish color indicates the conformation of carbohydrates as positive result (Ramakrishnan *et al.,* 1994).

(d) Test for Saponins

Saponins was confirmed in the selected medicinal plants in all extracts were confirmed by Kokate, (1999). 2 mL of each plant extract was added to 2mL of distilled water and shaken vigorously for 15 minutes in graduated cylinder lengthwise. The formation of 1cm layer of foam shows the confirmation of Saponins in the selected samples.

(e) Test for Cardiac- glycosides

For the detection of Cardiac-glycosides in the selected medicinal plants in all selected extracted solution. 0.5mL of each plant extract was added to 2mL of glacial acid and few drops of 5% ferric chloride .This was under layered with 1 mL of concentrated sulphuric acid. The appearance of brown ring at the interface shows the presence of cardiac glycosides in samples.

(f) Test for Terpenoids

An amount 0.5mL of each medicinal plant extract was added to 2mL of chloroform and concentrated sulphuric acid was added carefully to each extract of selected medicinal plants. The presence of red brown color at the interface indicates the presence of terpenoids in the selected medicinal plants.

(g) Test for Steroids

For the confirmation of steroids in the selected medicinal plants extracts.1mL of each plant extract in equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid in the sample. The appearance of brown ring indicates the presence of steroids in the selected medicinal plants.

(h)Test for Phytosteroids

An amount of 1mL of plant extract and equal volume of chloroform is added and subjected with few drops of concentrated sulphuric acid in the selected extracted solutions. The appearance of bluish brown ring indicates the presence of Phytosteroids in the selected medicinal plants.

(i)Test for Anthroquinones

For the confirmation of Anthroquinones in the selected plant extracts. 1mL of plant extract was added by a few drops of 10% ammonia solution, the presence of pink color precipitate indicates the presence of anthroquinones in the selected medicinal plants was in accordance Evans, (1997).

(j)Test for Phlobatanins

For the confirmation of Phlobatanins in the selected medicinal plants, 1mL of plant extract was added by few drops of 2% aqueous hydrochloric acid. The appearance of red color precipitate confirmed the positive result for of Phlobatanins in the selected medicinal plant extracts (Wagner, 1993; Evans, 1997).

(k)Test for Alkaloids

For the purpose of phytochemical analyses of selected medicinal plants, 2mL of plant extract, 2mL of concentrated hydrochloric acid was added. Then few drops of Mayer's reagent were added. The presence of green color or white creamy

precipitate indicates the positive results for alkaloids in the selected medicinal plan species.

(l)Test for Proteins

Selected medicinal Plant crude extract of 1mL and, 2mL of Million's reagent was added in the test tube. The appearance of white precipitate which turned into red upon gentle heating indicates the presence of protein in the selected medicinal plants (Gahan, 1984).

(j)Test for amino acids

For the detection of amino acids of the selected medicinal plants in the selected extract solutions. 5mL of plant extract was added by 2 drops of freshly prepared 0.2 per cent Ninhydrin reagent and heated. The appearance of blue color indicates the presence of amino acids in the selected medicinal plants (Yasuma & Ichikawa, 1953).

(k)Test for gums and mucilage

For the conformation of gums and mucilage in the selected medicinal plant species, 10mL of extract was added by 25mL of absolute alcohol under constant stirring. The appearance of precipitation indicates the presence of gums and mucilage in selected medicinal plants extracts (Maier *et al*., 1993).

(l) Test for fixed oils

For the conformation of fixed oil in the selected plants extracts. A small quantity of extract was passed between two filter papers. The appearance of an oily stain on the filter paper indicates the presence of oil in the selected plants extracts (Evans, 1989).

2.6 Quantitative phytochemical analysis

2.6.1Estimation of total Phenolic Content

Determination of total phenolic content in the selected medicinal plants was determined by using the standard method of (Singleton & Rossi, 1965; Haq *et al*., 2012). Before starting the practical we have prepared the stock solution through the following way.

2.6.2 Total phenolic content estimation

Total phenolic content in the selected medicinal plants was determined by using the following standard method of (Singleton & Rossi, 1965; Haq *et al*., 2012).

The total phenolic contents in selected medicinal plants in the following extract ethanol, acetone, chloroform and acetone was performed. The regent used in this experiment was FC, and Gallic acid was used as standard (positive control) 20 µL of each extract was transferred through Micropipette in to wells of 96 well micro titer plate followed by addition of 90 µL of FC reagent.

The micro titer plate along with mixture were kept at room temperature for 5 mints, 90 µL of sodium carbonate (6% w/v) was added to the wells carrying the mixture. The micro titer was then incubated for 30 mints at 37˚C and the absorbance of the mixture was recorded at 630 nm with the help of micro plate reader. This experiment was repeated in triplicate for correct data and confirmation, the calibration curve was drawn employing the same experimental conditions with Gallic acid (2.5, 5, 10, 20, 40 µg/mL) being used as positive control in this experiment. The total phenolic content (TPC) was calculated as µg Gallic acid equivalent (GAE) per mg of dry weight.

2.6.3 Total Flavonoids content estimation

The determination of total flavonoids content in the selected medicinal plants was done by using the aluminum chloride based calorimetric standard method (Boham & Kocipai-Abyazan, 1974).

The quercetin was used as positive control in this experiment. The test sample of 20 µL of each extract was transferred by micropipette in to the wells of 96 well micro titer plate followed by addition of 10 µL of 1 M potassium acetate, 10 µL of 10% (v/w) aluminum chloride and 160 µl distilled water. The micro titer plate was the incubated for 30 mints at room temperature, then the absorbance were measured at 415 nm by using the micro plate reader. In order to draw the calibration curve the quercetin at final concentrations of 2.5-10-20-40 µg/mL was used in this experiment. The resultant TFC was expressed in µg quercetin equivalent (QE) per mg dry weight and the experiment was repeated thrice for correct data.

2.6.4 Determination of total Flavonoids

The determination of total flavonoids content was done by using the standard method of (Boham & Kocipai-Abyazan, 1974; Haq *et al*., 2012).

10 gm. of each four selected medicinal plants powder were weighted and repeatedly extracted with 100 mL of 80% aqueous methanol at room temperature. Then the whole solution was extracted through Whitman filter paper No. 42. The filtrate was later transferred into the crucible and evaporated into dryness over water bath, weighted up to a constant weight obtained.

2.6.5 Total Alkaloids determination

The determination of alkaloid in the selected medicinal plants was carried out using the standard procedure of alkaline precipitation gravimetric method by (Harborne, 1973).Various plant extracts were prepared using different solvents includes ethanol, methanol, chloroform, and acetone, in each solution 5 gm of plants powder was weighted into a beaker of 250 mL and 200 mL of 10% acetic acid in solution and allowed to stand for 24 hours at room temperature. After 24 hours the mixture was filtrated through Whitman Filter paper (No.42) and then the extracts were concentrated on a water bath to ¼ Th of the original volume of the extract. Then concentrated ammonium solution was added in drop wise to the extract until the precipitation was completed. The whole solution was allowed to settle and the precipitated was collected and washed with 9% ammonium solution and then filtered. The residue obtained was alkaloid, dried in the oven at 60˚C for 30 mints and weighted. The process was repeated two more time and the average was taken. The weight of alkaloid was determined by the formula given below.

% of Alkaloid= (W2-W1)/ (Weight of sample) ×100

Where:-W1= Weight of filter paper

W2= Weight of filter paper with alkaloids precipitation

2.6.6Quantitive estimation of Saponins

The quantitive estimation of saponins in the selected extracted solution of medicinal plants was carried out by the method used by (Obadoni & Ochuko, 2001). 20 gm. of the powder sample was of each plant was put in to the conical flasks and 200 mL of 20% aqueous ethanol were added. The mixtures of each solution were shaken at shaker for 1 hour then the samples were heated on hot water bath for 4 hours at 55˚C. The mixture was filtered and the residue of each sample was re extracted with another 200 mL of 20% ethanol. The combined extracts were reduced to 40 mL over water bath at 90˚C. The concentrate was transferred into 250 mL separating funnel and 20 mL of di ethyl ether was added and were shaken strongly. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 mL of n-butanol was added. The combined n-butanol extracts were washed twice with 10 mL of 5% aqueous NaCl. The remaining solution was heated in a water bath. After evaporating the samples were dried in the oven to a constant weight; the saponins content were calculated in percentage.

% of Saponin= (W2-W1)/ (Weight of sample 1) ×100

Where: - W1= Weight of evaporating dish. W2= Weight of dish + Sample.

2.6.7Estimation of total carbohydrate content

Carbohydrate content in the selected extracts of medicinal was calculated by the standard method (Yemm & Willis, 1954) with some modification. The enthrone reagent was used in this method. 1.0 gm of each sample were homogenized in 10 mL of selected solvents of ethanol, chloroform, methanol and acetone and centrifuged at 500 rpm for 5 minutes. The supernatant were used for the estimation of total carbohydrate content. The reaction mixture consisted of 0.5 mL of supernatant and 5 mL of enthrone reagent which was boiled at 100 ˚C for 30 minutes. Absorbance was determined at 620nm. The carbohydrate content was expressed as µg/mg fresh weight. Data were statically analyzed by “SPSS” for graphical presentation of the obtained data.

2.6.8 Estimation of total protein content

Proteins estimation in the selected medicinal plants in all extract solutions was determined by standard method of Bradford, (1976). Before starting the experiment the following reagent was prepared for the practical. Coomassie brilliant blue G-250 (100 mg) was dissolved in 50 mL 95% ethanol then added 85% phosphoric acid w/v to 100 mL of solution. And the solution was diluted to a final volume of 1 liter. The final concentration in the reagent were 0.01% (w/v) Coomassie Brilliant blue G-250, 4.7% (w/v) ethanol, and 8.5% (w/v) phosphoric acid.

Solution containing 10-100 µg protein up to 0.1mL volume in the pipette into 12×100 mm test tubes. The volume of test tube adjusted up to 0.1 mL with the help of buffer.5 mL of proteins reagent was added to the test tube and the content mixed by inversion. The absorbance was measured at 595 after two mints and one hour in 3mL cuvettes against blank regent prepared from 0.1mL buffer solution and 5 Ml of protein reagent. And the protein weight was plotted against the absorbance resulting in a standard curve was used for the determination of the medicinal plants samples.

2.6.9Estimation of total tannins content

The determination of total tannins content in the various extracts, chloroform, acetone, ethanol and methanol of the selected medicinal was done by using the standard method of (Boham *et al.,* 1974; Van-Burden & Robinson 1981). 500 mg of powder of each plant were weighted into a 250 mL beaker. 50 mL of distal water was added and stirred for 1 hour on a mechanical shaker. The sample was filtered into a 50 mL volumetric flask and made up to the meniscus mark. Then the 5 mL of the filtered sample was measured into test tube containing 2 mL of 0.1 M FeCl3 in 0.1 M HCl and 0.008 M K3Fe (potassium

ferrocyanide). The absorbance was measured with a spectrophotometer at 120 nm wavelength within 10 minutes.

2.7Antimicrobial activity

3.6.1Plants collection

Among the documented medicinal plants we have selected only four medicinal plants, (Bunium persicum (Seeds), Olea ferruginea (fruits), Pistacia khinjuk (Leaves) Tulipa lehmanniana (Whole plants), for antimicrobial activity. The selected medicinal plants were collected from study area (district Harnai). Various plant parts were used in this study, fatherly these plant parts were brought into the laboratory, washed, dried under shades then grinded with the help of electric mortar in fine powder then passed through sieve of 40 meshes for further process.

3.6.2Sterilization of plant materials

The fresh, healthy and disease free plants parts of the selected medicinal plants were selected for the investigation. About 10 grams sample of each of plant were taken for each extracts preparation. Furthermore the surface was sterilized with 70 % alcohol for few seconds and then again the plants materials were washed thrice thoroughly with distilled water.

3.6.3Preparation of plant extract

10 gm powder of sterilized plant parts were kept in the 50 mL of each solvent Ethanol, Acetone, Methanol and Chloroform for extraction. The plants were kept on mover and shaker for further 24 hours to dissolve the constituents properly in the solvent, after 24 hours plants extracts were filtered, and then kept at room temperature for extraction. The extracts were kept in refrigerator for further antibacterial activity.

3.6.4 Selection of Bacterial Cultures

A total of eight bacterial strains, four (4) multidrug resistant and 4 environmentally collected bacterial strains were selected for the present research study. The bacterial strains were selected from the clinical setup.4 strains were gram positive and 4 strains were gram negative in present investigation. The clinically collected bacteria were Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus (MDR) (ATTC 25922), Staphylococcus aureus (ATTC 25923), and E.coli (ATTC 25922) was used as a positive control strains. Environmentally collected strains were (Microcrtus Lotus (ATTC 303099) Escherichia. coli (ATTC 434) , Staphylococcus aureus (ATTC 976), Enterobacter aerogenes (ATTC 13O48), Bacillus bravis (ATTC 1722) and Streptomycin … was used as positive control . and environmentally isolates biochemistry and molecular biology laboratory Biochemistry Department Quaid- I- Azam University Islamabad and all the above clinical isolates were collected from the Microbiology Department of Quaid -i-Azam University Islamabad Pakistan.

(Microcrtus lotus ATTC 303099 Escherichia coli (ATTC 434 – ve) , Staphylococcus aureus (ATTC 976 G+), Enterobacter aerogenes (ATTC 13O48 – ve), Bacillus bravis ATTC 1722 (+ve) ATTC 25923GC+, ATCCC 25922 GC- Klebsiella pneumonia (-ve), Escherichia coli (-ve), Staphylococcus aureus (MDR) (G+ve), Staphylococcus aureus (+ve) , the above all strains were collected from the Microbiology laboratory of Quaid-i-Azam University Islamabad Pakistan. four strains were gram positive and four strains were

3.6.5Preparation of Microbial inoculums

The fresh microbial culture was used for inoculums preparation. Nutrient Broth (NB) was prepared and poured into the test tubes, sterilized for 20 minutes at 121C temperature and 15 lbs pressure. Inoculation was done in the test tube by using sterile inoculating wire loop, incubated at 37°C for 24 hours. The 24 hours fresh culture was used for experimental analysis.

3.6.6Preparation of Nutrient Agar Medium

About 1.5 L of Nutrient agar medium was prepared and pH was adjusted at 6.8 while using pH meter by the addition of an alkali. The medium were sterilized in autoclave for 20 minutes at 15 lbs. pressure, and poured in the sterile petri plates.

3.6.7Screening for Antibacterial Activity Assay (Agar well diffusion method)

The antibacterial activities of each selected plant extracts were tested against the selected bacterial cultures. 25 mL of sterilized Nutrient agar medium was poured into each sterile petri plates and were allowed to solidify. Lawn of freshly bacterial culture was done through sterile cotton swabs. Then wells of 0.5 mm were made in the medium through sterile cork borer, followed by the addition of 150 µl of each ethanol, methanol, chloroform and acetone of each plant extracts were transferred in separate wells, plates were transferred to incubator for incubation at 37˚C for 24 hours. Ciprofloxacin antibiotic was used. After 24 hours of incubation plates were observed and the zone of inhibition of each well was measured in millimeter.

3.7 Statistical analysis

All the measurements were done in triplicate and statistical analysis were performed by using SPSS software and Microsoft excels 2007 and the results are presented in average.

3. RESULTS AND DISCUSSIONS

The present research is carried out to document the ethnobotanical data and to investigate the biochemical screening of the selected medicinal plants of district Harnai Balochistan province Pakistan. In this work total 234 wild plants species were reported for ethnobotanical uses includes their botanical name , local name, parts use, mode of decoction, disease cure / treated while phytochemical studies including qualitative analysis (carbohydrates, phenolic compounds, tannins, glycosides, anthroquinones derivatives, proteins, flavonoids, alkaloids, fixed oil , saponins ,amino acids, gums and mucilage) quantitative screening (total carbohydrates, proteins, phenols ,tannins, alkaloids, flavonoids and saponins) and antibacterial activity of the four selected medicinal plants in different extracts various solvents. The data regarding above motioned aspects is presented in the form of tables, figures graphs and plates.

Results and discussion

3.1 Phytochemical Analysis

Phytochemical results/ analysis of the following selected four medicinal plants

3.1.1 Qualitative phytochemical results of the selected medicinal plants

The qualitative phytochemical analyses of the selected four species are tested for only fourteen medicinally active constituents in this study. The phytochemical characters of the selected four medicinal plants investigated are summarized in the tables 6 and 7. The Qualitative phytochemical results for the selected medicinal plant species revealed that the flavonoids, sugar, protein, phenolic compounds, terpenoids, tannins, cardiac glycerides , steroids, alkaloids, amino acids were presents uniformly in all plants species in all four solvent extracts, glycosides are present in all four species with acetone solvent extracts and is absent in Tulipa, Pistacia and Olea with chloroform solvent extract and also with methanol extract in Tulipa and Pistacia , and it is also absent in Pistacia with methanol extracts. Anthroquinone is absent in Tulipa, Pistacia and Bunium with chloroform extract but are present in the rest of all extract solvent in all species, fixed oil and saponins is only absent in Tulipa with methanol extract and in Olea with ethanol extract solution while it is present with all the rest of solution extract in all four selected plant species. Carbohydrates are absent in acetone and ethanol extract solution in Tulipa, Bunium and Olea, and also absent with methanol extract solution in Bunium specie. Gums and mucilage are present in ethanol extracts in ethanol and methanol extract solution in all species while it is present in all the rest of extracts in all samples. As shown in the table no.2 and 3.

In the present study, the qualitative phytochemical analysis of selected four medicinal plants in the ethanol, methanol, acetone and chloroform extract solutions are studied for the first time in district Harnai Balochistan Pakistan. The results are quite unique and novel and reported for the first time on the medicinal flora of study area. There is no any such type of report on the phytochemical analysis of medicinal found in Balochistan and even in Pakistan to compare the results but a very five studies are found in the neighboring countries of Pakistan like India and Iran on the other aspects of the selected medicinal plants. (Fazly Bazzaz.*et a*l., 1997; Flamini *et al*., 2004; Shahsavari *et al*., 2008; Zangiabadi *et al.*, 2012; Bozorgi et al., 2013; Azadpour *et al*., 2015). So that our study is quiet unique and novel and reported on the quantitive phytochemical analysis for the first time on the selected medicinal plants in district Harnai Balochistan.

3.1.2 Quantitative phytochemical analysis of the selected medicinal plants

(a) Total percentage of carbohydrates

The determination of total carbohydrates in selected four medicinal plants was carried out on the popular method (Yemm & Willis, 1954) for the total determination of carbohydrates. The result is the mean values of three replicates are shown in the Table 8 and 9. The range of alkaloids percentage in all four plants is from 2.83-0.31µg/mg. Among the selected medicinal plants the Bunium persicum has the highest 2.83µg/mg of carbohydrates in the methanol solution and the lowest value was recorded for Tulipa lehmanniana 0.31µg/mg carbohydrates in the acetone solution. As shown in the table no.4 and 5.

In the present study the quantitive phytochemical analysis of selected medicinal plants of district Harnai Balochistan Pakistan in the ethanol, methanol, acetone and chloroform solutions are reported for the first time. The results are quite unique and novel. There is no any such type of such studies found in Balochistan and even in Pakistan on the above selected medicinal plants to compare the results but a very five studies are found in the neighboring countries of Pakistan like India and Iran on the other aspects of the selected medicinal plants.

(b) Total Flavonoids Content (TFC)

The result of Total Flavonoids Content (TFC) of all four selected medicinal plants in different extract i.e. ethanol, acetone, chloroform and methanol are shown in the table 8 and 9. The assay revealed that the highest flavonoids contents were reported in Tulipa lehmanniana in the acetone extract i.e. 16.32 µg/mg extracts. Expressed as mg quercetin equivalent per mg extract, it was followed by Bunium persicum ethanol extract and Pistacia khinjuk acetone extract 16.18 µg/mg for each and Pistacia khinjuk chloroform extract 16.05 µg/mg. The lowest TFC was reported for *Tulipa lehmanniana* 15.31 µg/mg in the chloroform extract. It has been reported from previous studies that flavoinds compounds have several pharmacological effects in the inhibition of arachidonic acid metabolism (Amresh *et al.,* 2007).In compression to the previous studies

conducted by other researchers on the on other aspects of the studied plants and other species of the same families for phytochemical analysis (Kala, 2005; Sharififar et al.,2010) studied the bioactive components of *Bunium persicum* and (Topçu *et al*., 2007) studied the acetone and methanol extracts of *Pistacia terebinthus* L. for their antioxidant activity, total phenolic and flavonoids contents of the fruit of this plants but our study is quite unique and novel and there is no previous reports of the same plants on studied aspects from Pakistan and particularly from Balochistan Harnai district. As shown in the table no.4 and 5.

In the present study the quantitive phytochemical analysis of selected medicinal plants of district Harnai Balochistan Pakistan in the ethanol, methanol, acetone and chloroform solutions are reported for the first time. The results are quite unique and novel. There is no any such type of such studies found in Balochistan and even in Pakistan on the above selected medicinal plants to compare the results but a very five studies are found in the neighboring countries of Pakistan like India and Iran on the other aspects of the selected medicinal plants. (Flamini et al., 2004)

(c) Total Phenolic Content (TPC)

The result of total phenolic content (TPC) of the selected four plants in different extract solutions i.e. ethanol, acetone chloroform and methanol are shown in the table 8 and 9. The results of the extracts assay revealed that the highest Phenolic contents are quantified in *Tulipa lehmanniana* in the chloroform extract i.e. 16.25 µg/mg extracts. Expressed as mg Quercetin equivalent per mg extract, it was followed by *Pistacia khinjuk* in chloroform extract 15.68µg/mg and *Olea ferruginea* in chloroform 15.25 µg/mg. As shown in the table no.4 and 5.

The lowest total flavonoids content (TPC) was reported in the *Tulipa lehmanniana* 11.5 µg/mg with ethanol extracts. In comparison to the studies conducted in Pakistan and around the world our result are quite different and unique from previous studies. The result of *Olea ferruginea* is quite different and unique with the results of Siddiqui *et al*, (2011). A number of studies shown that the variation in phenolic content is due to habit, altitude and other emissions. Sharma *et al*., (2012) reported that the medicinal plant (*Withania somnifera*) growing at road at road side containing more phenolic contents then those growing in the forests. And the same report was reported in Myrica esculenta due to altitude variation (Rawat *et al*., 2011). So that the results our study is different and reported for the first time on medicinal plants of district Harnai Balochistan Pakistan. There is no any type of literature found about the phytochemical analysis of *Tulipa lehmanniana* in the world. So that this the first ever reported from Pakistan regarding the phytochemical analysis of *Tulipa lehmanniana*.

(d) Determination of total % of alkaloids

The results of the total % of alkaloids of the selected four medicinal plants (*Bunium persicum, Olea ferruginea, Pistacia khinjuk and Tulipa lehmanniana*) in the all four extracts solutions ethanol, methanol, acetone and chloroform are shown in the table no 8 and 9. The results show that the *Tulipa lehmanniana* have the highest percentage of alkaloids in the methanol extracts (41.2%) and all the extracts show higher percentage of alkaloids of this plant species followed by *Olea ferruginea* with (32.9%) in the methanol extracts solution. The lowest percentage is reported in *Bunium persicum* with acetone extracts solution (10%). As shown in the table no.4 and 5. While in comparison with the previous phytochemical studies in Pakistan and in world on the medicinal plants (Siddiqui *et al.,* 2011; Bozorgi *et al.,* 2013), our results are quite unique and novel and reported for the first time in the selected medicinal plants of district Harnai Balochistan Pakistan.

(e) Determination of total % of Saponins

The results of the total % of Saponins of the selected four medicinal plants (*Bunium persicum, Olea ferruginea, Pistacia khinjuk and Tulipa lehmanniana*) in the all four extracts solutions ethanol, methanol, acetone and chloroform are shown in the table no8 and 9. The results show that the *Olea ferruginea* have the highest percentage of saponins in the ethanol extracts (15.2%) and all the extracts show higher percentage of Saponins of this plant species followed by Bunium persicum with (12.9%) in the methanol extracts solution. As shown in the table no.4 and 5.

The lowest percentage is reported in *Tulipa lehmanniana* with methanol extracts solution (4.9%). While in comparison with the previous phytochemical studies in Pakistan and in world on the medicinal plants (Siddiqui *et al*., 2011: Bozorgi *et al*., 2013) our results are quite unique and novel and reported for the first time in the selected medicinal plants of district Harnai Balochistan Pakistan. As shown in the table no.

(f) Determination of total of Proteins

The results of the total Proteins µg/ml of the selected four medicinal plants (*Bunium persicum, Olea ferruginea, Pistacia khinjuk and Tulipa lehmanniana*) in the all four extracts solutions ethanol, methanol, acetone and chloroform are shown in the table no 8 and 9. The results show that the *Bunium persicum* have the highest percentage of Proteins in the ethanol extracts (10.4µg/ml) and all the extracts show higher percentage of Proteins of this plant species followed by Tulipa lehmanniana with (7.2 µg/ml) in the ethanol extracts solution. The lowest percentage is reported in Pistacia khinjuk with chloroform extracts solution (10%). As shown in the table no.4 and 5.While in comparison with the previous phytochemical studies in Pakistan and in world on the medicinal plants (Siddiqui *et al*., 2011; Bozorgi *et al.,* 2013). Our results are quite unique and novel and reported for the first time in the selected medicinal plants of district Harnai Balochistan Pakistan.

3.2 Antibacterial activity of selected medicinal plants

The antibacterial activity of the selected four medicinal plants (*Bunium persicum, Olea ferruginea, Pistacia khinjuk and Tulipa lehmanniana*) from four different families in the ethanolic, methanolic, acetone and chloroform extracts solutions were selected due to their use for various medicinal purposes in district Harnai, these plant extract were screened for their antimicrobial activity specially in case of multi- drug resistant bacteria and environmentally collected bacteria these are *Staphylococcus aureus*(MDR) *Staphylococcus aureus, Klebsiella pneumoniae and Escherichia coli*, shown in table (6 and 7). And *Microcrtus Lotus, Escherchia.coli, Staphylococcus aureus and Enterobacter aerogenes* shown in the table No.6

The chloroform extracts of *Tulipa lehmanniana* shows a very good activity against *Staphylococcus aureus*(MDR) and *Escherichia coli* (-ve) bacteria, the acetone extracts of *Tulipa lehmanniana* has also shown very good activity against BG133-ve bacteria, while the rest of extracts of this plant shows no results against these multi-drug resistance bacteria. But this plant also shows very good result against the *Enterobacter aerogenes* (+ve) in the ethanol extracts. And in the rest of extract this plant shows no satisfactory results against any environmentally collected bacterial strains. The ethanolic, methanolic, acetone and chloroform extracts of *Pistacia khinjuk* have shown moderate results against *Staphylococcus aureus* (MDR) while there is no activity shown by the rest of all abstracts against these multi-drug resistance bacteria. This result is an is new and unique against these multi drug resistant bacterial strains .The previous studies conducted by other scientist in the world by the other parts of the Pistacia plant parts also shows moderate antibacterial activity by five Pistacia species grown in Iran by Bozorgi *et al.*,(2013). But our results regarding this plant are very satisfactory and the plant parts are used for the first time in such type of multidrug and environmentally collected bacteria. All the extract of selected plants shows very good results against the *Enterobacter aerogenes* (+ve) and moderate results against *Staphylococcus aureus*. And no results against other strains in any solutions. The methanolic and ethanol extracts of *Bunium persicum* have shown very good results against *Klebsiella pneumoniae* and the chloroform, ethanol and acetone extracts have moderate results against the *Staphylococcus aureus* (MDR) while no activity were shown against the rest of bacteria by any extracts. While ethanolic extracts of this plant show a very good result against the *Escherchia.coli*. Environmentally collected bacterial strains. The *Olea ferruginea* extracts in all solvents have shown no activity against any bacterial strains (MDR).While the ethanolic and acetone extracts show very good results against *Microcrtus Lotus* and *Staphylococcus aureus.*

In compression to the previous studies conducted on the antibacterial activates of medicinal plants in Pakistan and around the world. (Vala *et al.,* 2011) carried out the antibacterial study on the aerial parts of the selected medicinal plants from Iran. The extracts was tested against 4 Gram-positive and 5 Gram negative bacterial strains reported the antibacterial activity of E. persicus against S. aureus (MIC = 125 mg/ml), B. cereus (MIC =15.62 mg/ml), E. coli (MIC = 125 mg/ml), S. typhi (MIC = 31.25 mg/ml), S. dysantriae (MIC = 0.48 mg/ml) with a very good results for the first time. So that in our study a total of 16 ethanolic, methanolic, acetone and chloroform extracts of four medicinal plants of four different families from the district Harnai were tested against eight bacterial strains containing 8 Gram positive and 8 Gram negative bacteria. The selected plants have very important medicinal value in the district Harnai Balochistan and in the rest part of Pakistan and also in the world (Hajhashemi *et al*., 2011; Alamgeer *et al*., 2013; Bibi *et al* 2014). And no reports have been reported from Balochistan against any drug resistant bacterial strains of the selected medicinal plants .A very five studies are available on the antibacterial activity of these four medicinal plants in other parts of the world and no any drug resistant bacterial strains are tested in the previous studies conducted around the world and in Pakistan, in previous studies (Vala *et al.,* 2011)studied the antibacterial and antioxidant activity of *Pistacia lentiscus* and *Pistacia atlantica* leaves extracts against eight bacteria, five moulds and yeast and very weak antibacterial activity were observed in previous studies carried out on other species of the selected genera (Ghalem & Mohamed, 2009; Derwich *et al*., 2010). The number of extracts solutions were fever as compare to our study and also the plants parts which are used in the present study were not used before and no published data available on Tulipa lehmanniana in literature . This study was first time conducted on the medicinal flora of Harnai Balochistan. Three plants showed greater inhibitory activity in the methanolic extracts when compare to acetone, chloroform and ethanolic extracts. Bunium persicum methanolic extracts have shown very remarkable results against Klebsiella pneumoniae. It is the first study report in which the Bunium persicum have shown very good results against the bacterial strain *Klebsiella pneumoniae* in methanolic extracts for the first time from study area.

According to the present study it is concluded that plant extracts have great potential as an antimicrobial compounds against multi-drug resistance bacteria and environmentally collected bacteria and can be used as therapeutic agent against these pathogenic bacteria causing various infectious disease. *Bunium persicum* showed strong results against *Staphylococcus aureus* (MDR) and *Klebsiella pneumoniae* bacterial strains as compare to the earlier reports on the essential oil of *Bunium persicum* against other bacterial strains in Iran Talei & Mosavi,(2009) and also in compassion to the studies conducted by Ghderi P Ghderi *et al.,(*2014) in vitro antibacterial activity of the *Bunium persicum* and other plants against the Bacillus subtilis and *Staphylococcus aureus* by agar well diffusion methods and results of the study show that this may use against infectious bacteria. So there for our results are quite unique and novel regarding the medicinal plant *Bunium persicum* and this plant can be selected for further investigation and analysis. It can also be used to discover bioactive natural products that may lead towards the development of new pharmaceutical drug. Such natural organic compounds and identification of bioactive agents are the need of time and will play a vital role in the discovery and development of new and naval drugs.

4. CONCLUSIONS

The results of the phytochemical analysis and antibacterial activities against multiple drug resistance (MDR) and environmentally collected bacteria of the selected medicinal plants recommended that the claimed medicinal importance of the plants is due to the presence of large number of flavonoids, phenols, cardiac glycerides, saponins, glycosides, terpenoids, gum and mucilage, proteins, carbohydrates amino acids and fix oil which are all biologically active components. And the screened photochemical constituents seems to have the potential to use as source of drugs and also improve the health status of the users as result of the presence of various compounds that are necessary for good health. The antibacterial activities are due to the presence of above mentioned secondary metabolites. And the results of the selected medicinal plants show a remarkable activity against the multi drug resistant bacterial strains prove that these plants can be use as source of new drugs due to phytochemical constituents. Ethanol and methanol were the most effective solvent for extraction of the bioactive compounds. Furthermore the current results have motivated us to carry further studies on the characterization and isolation of the bioactive compounds of the above mentioned four plants in order to evaluate their mechanism and mode of action. The phytochemical studies results show the presence of the phytochemical constituents is important for research and manufacturing of new drugs for treatment of various diseases. The phytochemical properties identified by this study in medicinal plants of Harnai and their antibacterial activities will help in the preventing of different disease of this region and play an important role in the generation of new and nawal drugs.

5. RECOMMENDATIONS

To insure the sustainable use of medicinal plats wealth of the area.

Further biochemical investigation should be carried out on the medicinal flora of the area.

6. ACKNOWLEDGEMENT

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Table No.1. Medicinal uses of the selected plants by traditional people of Harnai.

Plant name	Local name	Family	Voucher	Collection place	Parts used	Disease treated/ traditional usage
Bunium persicum (Boiss.)	Tora zera.	Apiaceae	UOB-N-377	Chaperleft Harnai	Seeds	Digestive disorder, asthma, cold, cough and diuretic, diarrhea and dyspepsia
Olea ferruginea Royal.	Zathoon/ Shanelli	Oleaceae	UOB-N-378	Harnai Wham Thangi	Fruits	Used as ant diabetic, jaundice, typhoid, toothache, burning of eyes, scorpion sting, asthma
Pistacia khinjuk Stocks	Shiny	Anacardiaceae	UOB-N-379	Zarghoon Ghar	leaves	Antifungal, antiviral, antibacterial, antipyretic and anti-inflammatory agents and used as astringents for the treatment of diarrhea, disorder of liver, heart, kidney, respiratory system and throat infection
Tulipa lehmanniana Mercklin	Khatol and Gwarekh	Liliaceae	UOB-N-380	Zarghoon Ghar	Whole plant	Heart diseases, ointment for inflammation and also used as poultice, as make up in traditional cream for beauty

Table 2: Qualitative Phytochemical screening of plants in ethanol (E) and Methanol (M) solution

Plant species	Flav.[1*]		Glyc.[2*]		Sug.[3*]		Phen.[4*]		Terp.[5*]		Tan.[6*]		C. glyc.[7*]		Anthr.[8*]		St. and Phytos.[9*]		Alk.[10*]		A.A[11*]		F.O + Sap.[12*]		Prt.[13*]		Carb.[14*]	
	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M	E	M
Tulipa lehmanniana	+	+	++	–	+	+	++	++	+	+	++	++	+	+	+	+	+	+	+	+	+		++	–	+	+	–	+
Pistacia khinjuk	+	+	–	–	+	+	++	++	+	+	++	++	+	+	+	+	+	+	+	+	+	+	++	++	+	+	++	++
Olea ferruginea	+	++	++	++	+	+	+	++	+	+	++	++	+	+	+	+	+	+	+	+	+	+	–	+	+	+	–	+
Bunium persicum	+	+	++	++	+	+	+	++	+	+	++	++	+	+	+	+	+	+	+	+	+	+	++	++	+	+	–	–

1* = Flavonoids, 2* = Glycoside, 3* = Sugar, 4* = Phenols, 5* = Terpenoids, 6* = Tannins, 7* = Cardiac glycosides, 8* = Anthroqanins,
9* = Steroids and Phytosteroids, 10* = Alkaloids, 11* = Amino Acids, 12* =Fixed oils and saponins, 13* =Proteins, 14* = Carbohydrates

Table No. 3 Qualitative Phytochemical screening of plants in Chloroform (C) and Acetone (A) solution

Plant species	Flav.[1*]		Glyc.[2*]		Sug.[3*]		Phen.[4*]		Terp.[5*]		Tan.[6*]		C. glyc.[7*]		Anthr.[8*]		St. and Phytos.[9*]		Alk.[10*]		A.A[11*]		F.O + Sap.[12*]		Prt.[13*]		Carb.[14*]	
	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A	C	A
Tulipa lehmannian a	+	+	–	+	+	++	+	++	++	+	+	++	+	–	–	+	+	+	+	+	+	++	++	++	+	+	+	–
Pistacia khinjuk	+	+	–	+	+	++	+	++	+	+	+	++	+	–	–	+	+	+	+	+	+	+	++	++	+	+	++	++
Olea ferruginea	+	+	++	+	+	+	+	+	++	+	+	++	+	+	+	–	+	+	+	+	+	+	+	–	+	+	+	–
Bunium persic	+	+	–	+	+	+	+	+	++	+	+	++	–	+	+	+	+	+	+	+	+	+	++	++	+	+	+	–

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** – = Not present; + = Present in small quantity; ++= Present in large quantity

Table 4:Quantitative phytochemical analysis in Ethanol (E) and Methanol (M) Solution

Plant Name	Carbohydrates (µg/ml)		Flavonoids (µg/ml)		Phenols (µg/ml)		Alkaloids (%)		Saponins (%)		Proteins (µg/ml)	
	E*	M*	E	M	E	M	E	M	E	M	E	M
Tulipa lehmanniana	2.64	2.83	16.18	15.59	11.73	11.77	11	11.25	12.6	12.9	10.4	9.5
Pistacia khinjuk	2.71	2.73	15.48	15.94	14.69	14.86	32.8	32.9	15.2	14.4	6.3	6.0
Olea ferruginea	2.78	2.68	15.74	15.78	15.16	13.38	24	23	8.25	7.90	4.45	4.3
Bunium persicum	2.78	2.83	15.64	15.64	11.15	13.82	41	41.2	5.4	4.9	7.2	7.0

E* = Ethanol Solution; M* = Methanol Solution

Table 5: Determination of Proteins in the selected medicinal plants

Plant Name	Carbohydrates (µg/ml)		Flavonoids (µg/ml)		Phenols (µg/ml)		Alkaloids (%)		Saponins (%)		Proteins (µg/ml)	
	A*	C*	A	C	A	C	A	C	A	C	A	C
Tulipa lehmanniana	0.81	2.83	16.04	15.78	14.90	14.52	10	10.40	11.9	12.	9.7	9.9
Pistacia khinjuk	0.55	2.79	15.54	15.67	13.45	15.25	31	32.2	14.8	15	6.2	6.0
Olea ferruginea	2.18	2.24	16.18	16.05	14.51	15.68	22.5	23.9	8.00	8.51	4.56	4.0
Bunium persicum	0.31	2.29	16.32	15.31	13.41	16.25	40	40.23	5.6	5.1	6.9	6.7

A* = Acetone Solution; C* = Chloroform Solutio

Table 6: Zones of inhibition (mm) of crude extracts of Methanol, Ethanol Acetone and Chloroform of selected plants

Plant Name	*Staphylococcus aurous*				*Staphylococcus aurous* (MDR)				*Klebsiella pneumonia*				*Escherichia coli*			
	E*	M*	A*	C*	E	M	A	C	E	M	A	C	E	M	A	C
Tulipa lehmanniana	---	---	---	---	---	---	---	18	---	---	---	---	---	---	14	12

Pistacia khinjuk	---	---	---	---	11	11	11	14	---	---	---	---	---	---	---	---
Olea ferruginea	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---
Bunium persicum	---	---	---	---	---	13	14	15	16	20	---	---	---	---	---	---

Table 7: Zones of inhibition (in mm) of crude extracts of Methanol, Ethanol Acetone and Chloroform of selected plants

Plant Name	*Microcrtus lotus* (G+)				*Escherchia. coli* (G-)				*Staphylococcus aureus* (G+)				*Enterobacter aerogenes*				*Bacillus brevis*(G+)			
	E*	M*	A*	C*	E	M	A	C	E	M	A	C	E	M	A	C	E	M	A	C
Tulipa lehmannia na	---	6	---	6	---	4	---	4	4	6	---	---	14	6	---	---	---	10	---	4
Pistacia khinjuk	6	---	4	4	4	6	---	6	8	8	4	6	8	10	6	12	16	14	6	12
Olea ferruginea	14	---	---	6	---	---	---	---	10	---	12	---	4	8	---	4	8	12	---	---
Bunium persicum	4	6	---	6	20	4	---	---	4	---	---	---	6	4	---	---	6	4	10	6

E* = Ethanol Solution; M* = Methanol Solution; A*= Acetone Solution; C*= Chlorofrom Solut

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