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Phytochemistry and antibacterial activities of some selected plants of war affected area of bajaur agency, Pakistan

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Abstract
The Six medicinal plants species *Rumex dentatus* L., *Rumex hastatus* L., *Verbascum Thapsus* L, *Solanum nigrum* L., *Canabis sativa Linn*, and *Convolulus arvensis* L. were collected from the mountain of Arrang Sari ghar War affected area of Bajaur agency, Pakistan. The selected medicinal plants leaves were washed, air dried and then powdered. The methanolic, ethanolic and chloroform extract of leaf samples were used for the phytochemical investigation both (qualitative and quantative) and antibacterial activity. The key objective of the present work was to check the antibacterial activity and presence or absence of the phytochemical constituents of all the selected medicinal plants. The results of the phytochemical investigation of these medicinal plants showed that the alkaloids, Phlobatannins, tannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides and terpenoids were found to be present in all selected medicinal plants. Highest amount of phenol was found in the methanolic extract in sample *Rumex hastatus* (0.81±0.10 mg/g) followed by *Canabis sativa* (0.68±0.11 mg/g), *Convolulus arvensis* (0.58±0.20 mg/g), lowest amount of flavonoids was found *Solanum nigrum* (0.10±0.11 mg/g). Maximum zone of inhibition was detected against *Staphylococcus aureus* and *Salmonella typhi* with zone of inhibition of 24.17 and 21.00 mm respectively by *Rumex hastatus. Rumex hastatus* showed 17.00 mm zone of inhibition against *Escherichia coli.* The secondary metabolite of the plants is very essential commercially and has more concentration in pharmaceutical companies for the manufacture of the new drugs for curing of several diseases.

Keywords: *Qualitative phytochemistry, quantitative phytochemistry, antibacterial, bajaur agency, Pakistan*

1. Introduction
The medicinal plants are useful for human diseases as well as for curing of healing because of the presence of phytochemical constituents (Nostro *et al*., 2000) [22]. In the medicinal plants Phytochemicals are naturally present in roots, vegetables and leaves protect from various diseases due to their defense mechanism. Phytochemicals have two types. Primary and secondary constituents. Proteins, chlorophyll, and common sugars are incorporated in primary and secondary phytochemicals have alkaloids, terpenoids and phenolic compounds (Krishnaiah *et al*., 2000) [17]. Various important pharmacological activities exhibit by terpenoids i.e., anticancer, anti-malarial and anti-inflammatory inhibition of cholesterol production, anti-bacterial and anti-viral activities (Mahato & Sen, 1997) [18]. Terpenoids are very essential in attracting beneficial mites and ingest the herbivorous insects (Kappers *et al*., 2005) [14]. *Rumex dentatus* L belongs to the family Polygonaceae and is found all over the word throughout temperate region, 8 000–12 000 feet. It contains a large number of biologically active compounds and chemically complex and is traditionally used as bactericidal (Mothana *et al.,* 2010) [19], anti-tumor, astringent, anti-dermatitis, anti-inflammatory, diuretic, tonic, cholagogue, and laxative agents (Nisa *et al*., 2013) [21]. *Rumex hastatus*, belongs to the Polygonaceae family. The plant is suffrutescent richly branching shrub. It grows up to 90 to 120 cm tall and leaves with petioles of the same length as the blade; blade hastate, panicles terminal with erect-divergent, mostly simple branches, nut up to 2 mm long, brown, and long spindle-shaped roots. It is distributed in northern Pakistan, north east Afghanistan and south west of China, growing between 700 to 2500 m, sometimes grows as pure population (Qaiser, 2001). *Solanum nigrum* L are also important aspects of medicinal plant resources for treatment of primary health care. *Solanum nigrum* L commonly as Black night shade is a dicot weed in the Solanaceae family. It is an annual herbaceous plant of 10-60 cm high with a green, smooth and semi-climbing stem. The opposite leaves, with whole limb, oval and diamond shape are slightly cogged. It is a rather common species in wet woods, near river, waste land, old field,

ditches roadside and cultivated land (Gogoi & Islam, 2012) [9, 10]. *Verbascum thapsus* L. (Khardhag or Common mullein), a member of the family Scrophulariaceae, is a famous herb that is found all over Europe, in temperate Asia, in North America and is well-reputed due to its medicinal properties. It is famous in various communities worldwide for the treatment of various disorders of both humans and animals aliments. A number of pharmacological activities such as anti-inflammatory, antioxidant, anticancer, antimicrobial, antiviral and ant hepatotoxic (Riaz *et al*., 2013) [29]. *Cannabis sativa* is an annual plant belongs to the family Cannabaceae is commonly known as "Bhang", „Marijuana". It bears male and female flowers on separate plants because Cannabis is a dioecious plant. Cannabis sativa preparation is known by various names worldwide. It is called Marijuana in America; Bhang, Ganja and Charas in India; Kif in North Africa in some parts of South America (Sachindra & Pradhan). Cannabis is administered to patients suffering from rabies, cholera, rheumatism, epilepsy and tetanus. Also observation on the analgesic, anticonvulsive and muscle relaxant. *Cannabis sativa* have been used for the treatment of specific human ailments such as allergies, burns, cuts and wounds, inflammation, leprosy, leucoderma, scabies, smallpox and sexually transmitted diseases (Begum *et al.*, 2000) [3]. *Convolvulus arvensis* L. (Morning glory; family *Convolvulaceae*;) locally known as 'Leli' is a herbaceous perennial persistent, weed that is commonly found in regions with temperate mediterranean or tropical climates. since the eighteenth century it has use as herbal food and as a traditional medicine. in the salt ranges of Pakistan *C. arvensis* is commonly found and the inhabitants of the area are using for many generations this plant as folk medicine (Iqbal *et al*., 2011) [13].

2. Materials and methods

2.1 Plants collection and Botanical identification

The present study the plant species *Rumex dentatus* L., *Rumex hastatus* L., *Verbascum Thapsus* L, *Solanum nigrum* L., *Canabis sativa Linn*, and *Convolulus arvensis* L. these Six medicinal plants were collected from the mountain of Arrang Sari ghar War affected area of Bajaur agency, Pakistan. The collected plants were identified botanically in department of Botany, Abdul Wali Khan University Mardan Garden Campus. For the phytochemical analysis and anti-bacterial activity fresh leaves of selected plants were used.

2.2 Plant extracts Preparation

From the plants the leaves were removed and to remove dust then washed the selected plants under running tap water. For few days the plant samples were then air dried and into small pieces the leaves were crushed and stored in polythene bags. With the help of electric grinder the dried plant was powdered. The powder were kept in air tight plastic bottles for further phytochemical analysis. 10 gm of plant powdered was retained in distinct conical flask and 90 ml of solvent i.e. (methanol, ethanol and chloroform) was added to the powdered separately. With the help of aluminum foil the flask were covered and retained in shaker for 72 hrs for the shaking purposes. After 72 hrs the extracts were filtered with the help of Whatman filter paper and then through filtration process plant extracts were removed (Pirzada *et al.*, 2010) [27].

2.3 Phytochemical analysis

The plant extract i.e. methanol, ethanol and chloroform were tasted for the absence or presence of phytochemical constituents' like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides and terpenoids (Soni *et al.*, 2011) [35].

2.3.1 Alkaloids

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

2.3.2 Tannins

Ferric chloride test was used for the detection of tannins. Ferric chloride ($FeCl_3$) solution was mixed with all three (methanol, ethanol and chloroform) extracts separately. Formation of blue green coloration indicated the presence of tannins. (Kokate *et al*., 2008) [16].

2.3.3 Phlobatannins

In test tubes 0.5 ml of all the three extracts was taken separately, added 3ml distilled water and shaken for a few minutes then 1% aqueous hydro chloride (HCl) was added and boiled on water both. The presence of phlobatannins is indicated by the formation of red color (Wadood *et al.*, 2013) [38].

2.3.4 Flavonoids

For flavonoids detection, methanol, ethanol and chloroform extracts were treated with sodium hydroxide (NaOH) solution. red precipitation formation of indicate the presence of flavonoids (Kokate *et al.*, 2008) [16].

2.3.5 Carbohydrates

For detection of carbohydrates, 0.5 ml of all three extracts were treated with 0.5 ml of Benedict's regent. The solution were heated for 2 minutes on a water bath. By the formation of reddish brown precipitate the presence of carbohydrate was confirmed (Bussau, *et al*., 2002) [4].

2.3.6 Phenols

For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml methanol, ethanol and chloroform extracts in a test tube separately. Formations of deep bluish green solution showed the presence of phenol (Dahiru *et al.*, 2006) [7].

2.3.7 Saponins

For the detection of saponin, in test tube 5 ml of methanol, ethanol and chloroform extracts were shaken vigorously. The formation of froth indicated the presence of saponins (Rajesh *et al*., 2016).

2.3.8 Cardiac Glycosides

For cardiac glycosides detection, 2 ml of all three extracts solution were shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid (H_2SO4) and iron tri chloride ($FeCl_3$). The formation of a brown ring indicated the presence of glycosides (Soni *et al.*, 2011) [35].

2.3.9 Proteins

Xanthoproteic test: For the detection of protein, 1 ml of methanol, ethanol and chloroform extracts were treated with 1ml of concentrated nitric acid (HNO_3) solution. The presence

of proteins indicated by the formation of yellow color (Rajesh *et al.*, 2016).

2.3.10 Terpenoids

Salkowski test: One ml of methanol, ethanol and chloroform of plant extracts was added with 2 ml of chloroform and carefully added concentrated sulphuric acid (H_2SO_4) along the sides of tube to form a layer. By the formation of reddish brown coloration the presence of terpenoids was confirmed (Dahiru *et al.*, 2006) [7].

2.3.11 Glycosides

5% of Ferric chloride solution and 1 ml glacial acetic acid were added to 5 ml of all three extracts i.e. methanol, ethanol and chloroform and then further addition of few drops of concentered sulphuric acid (H_2SO_4). The presence of glycosides was conformed through the formation of greenish blue color (Rajesh *et al.*, 2016).

2.3.12 Quantitative analysis of total flavonoids and phenols Contents

2.3.13 Determination of total flavonoids contents

Methanol extracts were used for the detection of total flavonoids contents. Total flavonoids quantification was done by taking 0.5 g of plant extracts. Than the sample were mixed with 4.3 ml methanol and then more addition of 0.1 ml of aluminum tri chloride from 10% prepared solutions of aluminum tri chloride laterally. Potassium acetate (0.1 ml) was added the volume was reached to 5 ml. The mixtures were shaken by vortex to make uniform solution and then these mixture were placed at room temperature for 30 minutes for the purpose of incubation. After the completion of incubation process, the absorption was checked at 415 nm in spectrum. The Quercetin was used as a standard (Daffodil *et al*., 2013) [6].

2.3.14 Determination of Total Phenolic Contents

Total phenolic quantification was done by the addition of 0.5g plant extract to 1 ml of 80% ethanol. Then the mixture were centrifuged for 15 minutes at 12,000 rpm. After that the supernatant were kept in test tube and these process were repeated 6 times. After collecting the supernatant were placed in water bath for drying. The distilled water was added to the supernatant until its volume reached to 3 ml. 2 ml (Na_2CO_3) of 20% were added in this solution. To this 0.5 ml Folin - ciocalteau regent was added and after 5 minutes more addition of 2 ml (Na_2Co_3) from 20% Na_2Co_3 solutions. The solution were mixed homogenously and then the test tube were brought in to the water bath in boiling water. At 650 nm their absorbance were checked. The Catechol was used as a standard (Hagerman *et al*., 2004) [11].

2.3.15 Determination of Total Tannin contents

Methanol extracts were used for the detection of total tannin contents. 500 mg of the sample was weighed into a 50 ml plastic bottle. 50 ml of distilled water was added and shaken for 1 h in a mechanical shaker. This was filtered into a 50 ml volumetric flask and made up to the mark. Then 5 ml of the filtered was pipetted out into a test tube and mixed with 2 ml of 0.1 M FeCl3 in 0.I N HCl and 0.008 M potassium Ferro cyanide. The absorbance was measured at 120 nm within 10 min. (Van & Robinson, 1981).

2.4 Antibacterial Activity

2.4.1 Crude extracts Antibacterial

To screen the antibacterial activity of the selected medicinal plants chloroform, methanolic and ethanolic extracts the agar well diffusion method was used (Perez *et al*., 1990) [26]. With crude extracts of all plants the assay was performed.

2.4.2 Microorganisms used

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

2.4.3 Media for bacterial culture

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

2.4.4 Inoculum Preparation

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

2.4.5 Agar Plates Preparation

At room temperature LB agar was left to cool, it was poured into sterilized petri plates before to solidify. The agar well diffusion method (Perez *et al*., 1990) [26] was used. Using a sterile cotton swab cultures lawn of the test organisms were made on the agar plates. Using a sterile borer under sterile conditions five wells were made per plate.

2.4.6 Extract Preparation for activity

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

2.4.7 Measurement of zone of inhibition and Pouring of test solution incubation

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

Statistical analysis

All the tests were performed as individual triplicate experiment. All the data are expressed as mean ± standard deviation.

Results and Discussion

3. Phytochemical analysis

In the present research work the both qualitative and quantative phytochemical investigation of methanolic, ethanolic and chloroform extracts of *Rumex dentatus* L.,

Rumex hastatus L., *Verbascum Thapsus* L, *Solanum nigrum* L., *Canabis sativa Linn*, and *Convolulus arvensis* L. and antibacterial activity was carried out.

3.1 Qualitative Detection of Bioactive compound in the leaves of the plants

Qualitative analysis of *Rumex dentatus* L., *Rumex hastatus* L., *Verbascum Thapsus* L, *Solanum nigrum* L., *Canabis sativa Linn*, and *Convolulus arvensis* L. was carried out for the detection of alkaloid, flavonoids, carbohydrate, phlobatannins, glycosides, saponins, phenol, terpenoids, tannins, cardiac glycosides and proteins. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins and glycosides were found in the chloroform extracts. Flavonoids, carbohydrates, saponins, phenols, terpenoids and protein were found present in chloroform extracts. In these results +++ indicate that the secondary metabolites present in highest amount, the ++ indicated that the moderate level of phytochemicals' are present and the + indicated that low level of phytochemicals are present in all these three extracts of all the six plants (Table 1, 2 and 3).

Table 1: Qualitative Detection of Bioactive compound in the leaves of the selected medicinal plants methanolic extracts

S. No	Phytochemical test	*Rumex dentatus*	*Rumex hastatus*	*Verbascum Thapsus*	*Solanum nigrum*	*Canabis sativa*	*Convolulus arvensis*
1	Alkaloid	++	+	+++	+	++	+
2	Flavonoids	+	+++	+	+++	+	++
3	Carbohydrate	+++	+	+++	+	+	++
4	Phlobatannins	+	++	+++	+	++	+
5	Glycosides	++	+++	+	+	+++	+
6	Saponins	+++	+++	+	+++	+	+
7	Phenol	+	+	+++	+++	+	+
8	Terpenoids	+	+++	+	++	++	+
9	Tannins	+++	++	+	+++	+	+++
10	Cardiac glycosides	++	+	+++	+	++	+++
11	Proteins	++	+	+++	+	++	++

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

Table 2: Qualitative Detection of Bioactive compound in the leaves of the selected medicinal plants in ethanolic extracts

S. No	Phytochemical test	*Rumex dentatus*	*Rumex hastatus*	*Verbascum Thapsus*	*Solanum nigrum*	*Canabis sativa*	*Convolulus arvensis*
1	Alkaloid	+++	+	+++	+	++	++
2	Flavonoids	+	+	++	+++	+	++
3	Carbohydrate	+	++	+++	+	+++	+
4	Phlobatannins	++	++	+++	+	+	+
5	Glycosides	+	++	+	+++	+	++
6	Saponins	+++	+	+	++	+++	+
7	Phenol	+	+++	+	+	+	+
8	Terpenoids	+	+++	+	+++	++	++
9	Tannins	+	+++	++	++	+	+++
10	Cardiac glycosides	+++	+	+++	++	++	+
11	Proteins	+	++	++	+++	+	+

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

Table 3: Qualitative Detection of Bioactive compound in the leaves of the selected medicinal plants in chloroform extracts

S. No	Phytochemical test	*Rumex dentatus*	*Rumex hastatus*	*Verbascum Thapsus*	*Solanum nigrum*	*Canabis sativa*	*Convolulus arvensis*
1	Alkaloid	+	++	+	+++	+	++
2	Flavonoids	+	++	++	+	++	+
3	Carbohydrate	++	+++	++	+	+++	+++
4	Phlobatannins	+++	+	+++	+	++	+
5	Glycosides	++	+	++	++	+++	+
6	Saponins	+++	+++	+	+++	+	++
7	Phenol	+	+++	+	+++	++	+
8	Terpenoids	++	++	++	+++	+++	+
9	Tannins	+++	+	+++	++	+	+++
10	Cardiac glycosides	+	+++	++	+	+++	++
11	Proteins	+	+++	++	++	+++	+

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

3.2 Qualitative Phytochemistry of selected medicinal plants in methanolic extracts

The selected medicinal plants were comparatively studied for their total flavonoids contents, total phenolic and tannin compounds in the solvents methanol. (The Quercetin were used as a standard for flavonoids having y = 0.0031× + 0.0159 while the value of R^2 = 0.9997. The Catechol was used as a standard for phenol and tannin having the values of R^2 = 0.999 y= 0.0012 × + 0.0659). Highest amount of phenol was found in the methanolic extract in sample *Rumex hastatus*

(0.81±0.10 mg/g) followed by *Canabis sativa* (0.68±0.11 mg/g), *Convolulus arvensis* (0.58±0.20 mg/g), lowest amount of flavonoids was found *Solanum nigrum* (0.10±0.11 mg/g).The highest amount of flavonoid was determined in methanolic of *Solanum nigrum* (0.98±0.10 mg/g), followed by *Rumex hastatus* (0.96±0.10mg/g), *Canabis sativa* (0.77±0.11mg/g), *Convolulus arvensis* (0.61±0.01mg/g) and lowest amount of flavonoid was found in *Verbascum Thapsus* (0.15±0.2mg/g). The highest amount of was determined in methanolic of *Convolulus arvensis* (15.25±0.11mg/g), followed by *Convolulus arvensis* (15.05±0.14mg/g), *Rumex hastatus (*11.85±0.31mg/g), *Verbascum Thapsus* (9.98±0.32 mg/g), *Canabis sativa* (7.45±0.22 mg/g) and lowest amount of tannin was found in *Solanum nigrum* (6.08±0.23 mg/g). The data showed in table 4.

Table 4: Qualitative Phytochemistry of selected medicinal plants in methanolic extracts

S. No	Plants name	Total phenol contents mg/g	Total flavonoid contents mg/g	Total Tannin contents mg/g
1	*Rumex dentatus*	0.20±0.20	0.34±0.20	15.25±0.11
2	*Rumex hastatus*	0.81±0.10	0.96±0.10	11.85±0.31
3	*Verbascum Thapsus*	0.14±0.23	0.15±0.2	9.98±0.32
4	*Solanum nigrum*	0.10±0.11	0.98±0.10	6.08±0.23
5	*Canabis sativa*	0.68±0.11	0.77±0.11	7.45±0.22
6	*Convolulus arvensis*	0.58±0.20	0.61±0.01	15.05±0.14

3.3 Antibacterial activity of crude ethanolic extracts of selected medicinal plants

The results of antibacterial activity of crude ethanolic extracts showed in the (table 5) that the selected medicinal plants were active against the bacterial strains. Maximum zone of inhibition was observed against *Staphylococcus aureus* and *Salmonella typhi* with zone of inhibition of 24.17 and 21.00 mm respectively by *Rumex hastatus. Rumex hastatus* showed 17.00 mm zone of inhibition against *Escherichia coli, Convolulus arvensis* showed 19.00 mm zone of inhibition against Staphylococcus aureuss.15.67mm inhibition against Salmonella typhi while the *Verbascum Thapsus showed* 15.30mm inhibition against *Escherchia coli*. And the *Solanum nigrum* showed 17.00mm inhibition agonist the *Pseudomonas aeroginosa*. The *Canabis sativa* showed 19.00mm inhibition against *Staphylococcus aureus and* 18.33mm inhibition against *Escherchia coli.*

Table 5: Antibacterial activity of crude ethanolic extracts of selected medicinal plants

Plant	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeroginosa*	*Escherchia coli*	*Salmonella typhi*
Rumex dentatus	3.57± 0.83	5.89± 2.47	4.30± 0.35	3.58± 1.21	2.12± 0.12
Rumex hastatus	24.17± 0.73	15.75± 0.69	16.74± 0.63	17.00± 1.00	21.00± 0.58
Verbascum Thapsus	13.00± 0.58	11.67± 0.88	13.41± 0.46	15.30± 0.73	13.17± 0.142
Solanum nigrum	15.00± 0.32	12.67± 0.51	17.00± 0.43	13.67± 0.67	14.33± 0.53
Canabis sativa	17.67± 0.34	7.67± 0.67	13.63± 0.61	15.00± 0.56	10.46± 0.34
Convolulus arvensis	19.00± 0.56	13.67± 0.54	14.67± 0.34	18.33± 0.78	15.67± 0.56

3.4 Antibacterial activity of crude methanolic extracts of selected medicinal plants

The results of antibacterial activity crude methanolic extracts was display in the (table 6) that the selected plants were active against the bacterial strains. Maximum zone of inhibition was observed against *Escherchia coli* and *Shigella flexneri* with zone of inhibition are 19.00 mm and 17.75 mm respectively by *Rumex hastatus. Verbascum Thapsus* showed 17.17 mm zone of inhibition against, *Salmonella typhi* and *Convolulus arvensis* showed 19.33 mm zone of inhibition against *Escherchia coli*. 15.67mm inhibition against Salmonella typhi and the *Solanum nigrum* showed 17.67 mm inhibition agonist the *Shigella flexneri.* The *Canabis sativa* showed 14.46 mm inhibition against *Salmonella typhi* and 13.00 mm inhibition against *Escherchia coli.*

Table 6: Antibacterial activity of crude methanolic extracts of selected medicinal plants

Plant	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeroginosa*	*Escherchia coli*	*Salmonella typhi*
Rumex dentatus	9.57± 0.73	8.89± 1.47	6.30± 0.39	8.58± 1.28	4.12± 0.16
Rumex hastatus	19.17± 0.77	17.75± 0.59	13.74± 0.67	19.00± 1.00	16.00± 0.58
Verbascum Thapsus	10.00± 0.58	13.67± 0.88	16.41± 0.46	14.30± 0.73	17.17± 0.142
Solanum nigrum	14.00± 0.32	17.67± 0.51	13.00± 0.43	15.67± 0.67	16.33± 0.53
Canabis sativa	15.67± 0.34	8.67± 0.67	11.63± 0.61	13.00± 0.56	14.46± 0.34
Convolulus arvensis	13.00± 0.56	11.67± 0.54	15.67± 0.34	19.33± 0.78	10.67± 0.56

3.5 Antibacterial activity of crude chloroform extracts of selected medicinal plants

The results of antibacterial activity of chloroform extracts was display in the (table 7). The selected plants were active against the bacterial strains. Maximum zone of inhibition was observed against *Salmonella typhi* and *Escherchia coli* with zone of inhibition are 20.33 mm and 18.67 mm respectively *Solanum nigrum. Verbascum Thapsus* showed 19.17 mm zone of inhibition against, *Salmonella typhi* and *Convolulus arvensis* showed 18.00 mm zone of inhibition against *Staphylococcus aureus.* 17.33 mm inhibition against *Escherchia coli* and the *Rumex hastatus* showed 18.00 mm inhibition agonist the *Escherchia coli.* The *Canabis sativa* showed 18.46 mm inhibition against *Salmonella typhi* and 15.00 mm inhibition against *Escherchia coli.*

Table 7: Antibacterial activity of crude chloroform extracts of selected medicinal plants

Plant	*Staphylococcus aureus*	*Shigella flexneri*	*Pseudomonas aeroginosa*	*Escherchia coli*	*Salmonella typhi*
Rumex dentatus	6.57± 0.83	5.89± 2.47	4.30± 0.35	6.58± 1.21	7.12± 0.12
Rumex hastatus	16.17± 0.73	13.75± 0.69	17.74± 0.63	18.00± 1.00	14.00± 0.58
Verbascum Thapsus	13.00± 0.58	15.67± 0.88	18.41± 0.46	16.30± 0.73	19.17± 0.142
Solanum nigrum	10.00± 0.32	15.67± 0.51	14.00± 0.43	18.67± 0.67	20.33± 0.53
Canabis sativa	13.67± 0.34	7.67± 0.67	14.63± 0.61	15.00± 0.56	18.46± 0.34
Convolulus arvensis	18.00± 0.56	14.67± 0.54	12.67± 0.34	17.33± 0.78	11.67± 0.56

Discussion

In the present research work the qualitative investigation of methanolic, ethanolic and chloroform extracts of *Rumex dentatus* L., *Rumex hastatus* L., *Verbascum Thapsus* L, *Solanum nigrum* L., *Canabis sativa Linn*, and *Convolulus arvensis* L. was carried out. The results showed that alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins and cardiac glycosides was found in methanolic and ethanolic extracts, while alkaloids, phlobatannins and glycosides were found in the chloroform extracts. Flavonoids, carbohydrates, saponins, phenols, terpenoids and protein were found present in chloroform extracts. The Flavonoids, carbohydrates, saponins, phenols, terpenoids and protein were present in highest amount in methanolic, ethanolic and chloroform extracts. The alkaloids, flavonoids, carbohydrates, phlobatannins, saponins, phenols, terpenoids, tannins, cardiac glycosides were present in *Rumex hastatus* and *Verbascum Thapsus* in highest amount and *Solanum nigrum* L., *Canabis sativa Linn*, and *Convolulus arvensis* in moderate levels. In chloroform extracts Carbohydrate, Phlobatannins, Saponins, tannins and Cardiac glycosides were presents in highest amounts in *Rumex hastatus, Canabis sativa* and *Rumex dentatus* and moderate levels in *Verbascum Thapsus* and *Solanum nigrum.* Maximum zone of inhibition was observed in chloroform extracts against *Salmonella typhi* and *Escherchia coli* with zone of inhibition are 20.33 mm and 18.67 mm respectively *Solanum nigrum.* In methanolic extracts maximum zone of inhibition was observed against *Escherchia coli* and *Shigella flexneri* with zone of inhibition are 19.00 mm and 17.75 mm respectively by *Rumex hastatus. Verbascum Thapsus* showed 17.17 mm zone of inhibition against, *Salmonella typhi* and *Convolulus arvensis* showed 19.33 mm zone of inhibition against *Escherchia coli* in ethanolic extracts observed against *Staphylococcus aureus* and *Salmonella typhi* with zone of inhibition of 24.17 and 21.00 mm respectively by *Rumex hastatus. Rumex hastatus* showed 17.00 mm zone of inhibition against *Escherichia coli, Convolulus arvensis* showed 19.00 mm zone of inhibition against *Staphylococcus aureus.* The phenolic compounds are the groups of plant metabolites (Singh *et al*., 2007) [32]. They possess biological properties such as, antiaging, ant inflammation, and cardiovascular protection (Han, *et al*., 2007) [12]. Flavonoids are wide range of phytochemical with various pharmacological effects including antioxidant, anti-inflammation, anti-platelet, anti-allergic, cytotoxicity, reduce risk for heart disease or cancer etc (Asif *et al.,* 2013) [2]. Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidant cell damage have strong anticancer activity (Okwu *et al*., 2007, Salah *et al*., 1995) [24, 31]. Flavonoids in intestinal tract lower the risk of heart disease. As antioxidants, flavonoids from these plants provide anti-inflammatory activity (Okwu, 2004) [23]. The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation. Saponins have the property of precipitating and coagulating red blood cells. Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al*., 2000) [34]. The saponins are used in hypercholesterolemia, hyperglycemia, antioxidant, anticancer, anti as inflammatory activity and weight loss (Manickam murugan *et al*., 2014) [20]. Saponins act as antimicrobial activity and extremely cold blooded animals, but toxicity to mammals is low (Verma *et al*., 2013) [37]. Alkaloids have the analgesic, antispasmodic and antibacterial (Deshmukh *et al*., 2004) properties. Alkaloids have been used as both antibacterial and antidiabetic properties and useful for such activities. Phenols and phenolic compounds have been extensively used in disinfections and remain the standard with which other bacterisides are compared (Akinyeye *et al.,* 2014) [1]. Glycosides are known to lower the blood pressure. Tannins are also known antimicrobial agent. Tannins (commonly referred to as tannic acid) are water soluble polyphenols that are present in many plant foods. Tannins are water soluble plant polyphenols that precipitate proteins. Tannins have been reported to prevent the development of microorganisms by precipitating microbial protein and making nutritional protein unavailable for them (Sodipo *et al*., 1991) [33]. The growth of many fungi, yeasts, bacteria and viruses was inhibited by tannins (Chung *et al*., 1998) [5]. Phytotherapatically tannin containing plants are used to tract nonspecific diarrhea, inflammations of mouth and throat and slightly injured skins (Westendary, 2012). Variety of proteins have been isolated in medicinal plants and found to be bioactive against certain ailments (Tsao *et al*., 1990).

Conclusion

The selected six medicinal plants are the main basic source of the phytochemicals i.e., alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides and terpenoids. Medicinal plants play a key role in preventing several diseases. The anti-bacterial, anti-inflammatory, analgesic, antidiuretic, anti-viral, anticancer, anti-malarial and anti-fungal activities of the medicinal plants are due to the presence of the above present phytochemicals. Medicinal plants are used for screening and discovering of the secondary metabolites which are very useful for the production of new medicine. The phytochemical analysis of the medicinal plants are also important and have commercial interest in both pharmaceuticals companies and research institutes for the formation of the new medicine for treatment of several diseases. Thus we hope that the important phytochemical properties identified by our study in the local plant of Arrang Sari ghar War affected area of Bajaur agency, Pakistan will be helpful in the copping different diseases of this particular region.

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