

Original Article

# Qualitative and quantitative assessment of Phytochemicals in selected Medicinal Plants native to Shujabad, Punjab, Pakistan.

Shazia Jabeen<sup>1\*</sup>, Nazia Amir<sup>2</sup>, Syeda Shaima Meryam<sup>1</sup>

<sup>1</sup> Department of Environmental Sciences, The Women University Multan, Pakistan

<sup>2</sup> Department of Botany, The Women University Multan, Pakistan

\* Correspondence: shazia.jabeen@wum.edu.pk

**Abstract:** The present study aimed at screening of phytochemicals qualitatively and quantitatively in six selected medicinal plants native to Shujabad, Multan, Pakistan. Screening of various phytochemicals (phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, anthocyanins, phlobatannins, coumarins, triterpenoids, cardiac glycosides, sterols and alkaloids) was done by preparing plant leaf extracts in different solvents i.e., ethanol, methanol and water.

Optical density of total phenolic and alkaloid compounds was measured via UV-Visible spectrophotometer at 640 nm and 470 nm respectively. Phenols and anthocyanins were found in all the six selected medicinal plants. Most of plants have the studied phytochemicals. *C. citratusis* and *C. album* showed the presence of 13 studied phytochemicals. Phenolic content was found highest in *R. communis* while highest alkaloid content in *E. prostrate* was found. It is evident from the study that *C. citratusis* of highest therapeutic efficacy possessing majority of phytochemicals classes of compounds and *O. corniculata* of lowest therapeutic potential due to the absence of majority of phytoconstituents. Plants having these phytochemicals are considering important both medicinally and economically. There is need to do further research on phytoconstituents of these plants and their role in mitigating the disease.

**Keywords:** Shujabad, Phytochemicals, TPC, TAC

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## 1. Introduction

Plants have been the source of drugs but now-a-days they are preferred over conventional medicines owing to their easy accessibility, low cost and effectiveness [1]. Biologically active, naturally occurring phytoconstituents of help in protection against the diseases in humans and also contribute in adding micro and macronutrients [2]. Regular uses of phytochemicals in daily diet are of great benefit for human health. Primary and secondary metabolism is responsible for the preparation of phytochemicals in living beings [3].

For the cure or prevention of dangerous diseases, many health care programs has been made but excessive and long term usage of allopathic medicines leads to harmful side effects [4,5]. Educated and well aware communities prefer plant based medicines owing to their effective results and marginal side effects. All of this is because these

medicines showed fewer side effects, available at low cost and showed comparison with physiological flora [4, 6].

Phytoconstituents naturally present in plants give the colour and other qualities to plants. They had the value as essential nutrient and having the biological significance in plants [7]. About 96 % phytochemicals showed biological activities obtained from plant extracts as biological active constituents. A large number of bioactive elements found in fruits and herbs which used for various purposes and save plants itself from harsh environmental conditions [8].

Plants have the phytochemicals which save the plants from any harm and give the scent, colour and taste. They play significant role to protect the plants from various types of changes in environment such as pollution, drought, and exposure to UV rays and pathogenic attack [9].

The present study focuses on the qualitative examination of phytochemicals and quantitative estimation of the total phenol and total alkaloid content in selected medicinal plants native to Shujabad area, Punjab Pakistan.

## 2. Materials and Methods

### 2.1. Study Area

Shujabad is a tehsil of Multan District. It is situated about 45 km from Multan. Chenab River is also placed in its locality [10] (Figure. 1). The area around the city is fertile. The land of this area has become ideal for agriculture because of irrigation from freshwater of canal. Main fruits grown in Shujabad are mangoes, dates, citrus, guavas, black plum, pears, black currant, Assyrian plum and banana while main vegetables are potatoes, onion and cauliflower [11].

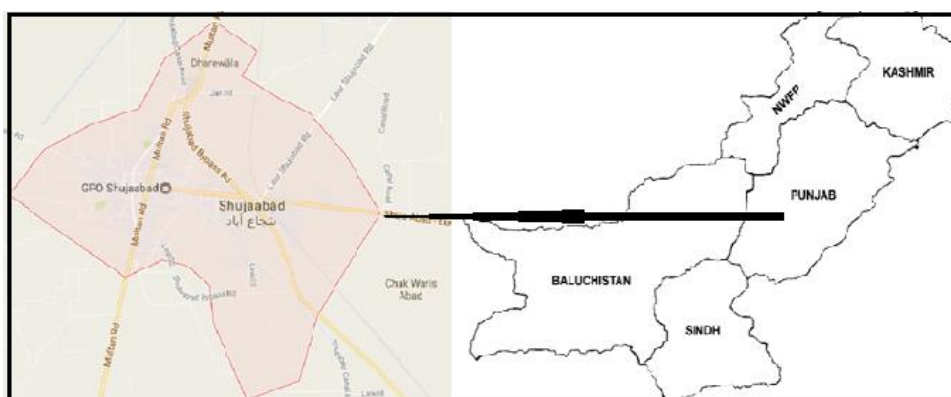


Figure 1. Location Map of study area

## 2.2. Sampling and Processing

Six medicinal plants were selected from study area for screening their phytochemicals (Table.1). The plants were uprooted along with leaves and stems from the study area in August and September. The samples were then transported to Department of Botany lab at WUM, Multan for further processing. Plant samples were washed thrice with water to get rid of dust particles and insects. The leaves were dried under shade about 3-4 weeks. The dried leaves were grinded by electric grinder to get fine powder. Powdered plant materials were stored in airtight glass containers to protect from moisture during qualitative and quantitative phytochemical analysis.

**Table 1: Scientific names of selected plant species with family names**

Sr.No	Species name	Family	Habit
1	<i>Chenopodium album</i>	Chenopodiaceae	Leaves
2	<i>Ricinus communis</i>	Euphorbiaceae	Leaves
3	<i>Oxalis corniculata</i>	Oxalidaceae	Leaves
4	<i>Mentha piperita</i>	Lamiaceae	Leaves
5	<i>Cymbopogon citratus</i>	Poaceae	Leaves
6	<i>Eclipta prostrata</i>	Asteraceae	Leaves

## 2.3. Extraction of Plant Materials

About 15g of dried powder of each sample were soaked in chloroform in three separate beakers. 100 ml methanol, 100 ml ethanol and 100 ml distilled water were added to each beaker separately. After shaking for 10 minutes, the soaked material were filter through Whatman filter paper # 40. The filtrate was evaporated by using rotary evaporator at 45°C till the required dryness achieved. The whole experiments were run in triplicate. These extracts were stored in vials at 4°C for further analysis.

## 2.4. Calculation of % extraction yield

The amount of extracted material calculated by formula reported by Mandal, 2013 [12].

Amount of Extract (mg) = Weight of dry extract with petridish- Weight of petridish

% extract yield = Dry weight of extract/Weight of powdered material × 100

## 2.5. Qualitative analysis of phytochemicals in plant extract

Qualitative determination of secondary metabolites i.e., Phenols, Alkaloids, Anthocyanins, Flavonoids, Tannins, Phlobatannins, Coumarins, Terpenoids, Triterpenoids, Steroids, Sterols, Saponins, Glycosides and cardiac glycosides were done by using their aqueous extracts [13]. The evaluation of alkaloids was performed by a procedure described by Chakraborty (2010) [14] while the flavonoids were determined by test as described by Butterweck et al., 2000 [15]. The presence of saponin was determined by the Froth test [16] while phenol and tannin contents were determined using ferric chloride test [14]. The determination of steroids, sterols, terpenoids, triterpenoids, were done using Salkowski test. The glycosides were detected by Liebermann's test and Salkowski's test and the cardiac glycoside content was determined by the Keller-Killani's test [17]. Finally, the presence of coumarins was determined using sodium hydroxide and Precipitate test was performed for the detection of phlobatannins [18].

## **2.6. Quantitative phytochemical screening of plant extract**

### **2.6.1. Estimation of Phenolic Contents**

The phenolic contents of extracted samples were analyzed by Folin-ciocalteu method. To prepare the organic extracts of plant samples, about 1 gm of plant extracts were combined with 20 ml of 7:7:6 (volume/volume) of acetone, methanol and distilled water respectively. The extracts were centrifuged at 6000 rpm for 10 minutes. The phenolic contents of organic extract were analyzed by using reported methodology [19, 20]. About 10ml of distilled water and 2ml of Folin-ciocalteu reagent was added in 1 ml of organic extract. Two ml of saturated sodium carbonate solution was added in the prepared mixture and incubated at ambient temperature in dark for one hour. After the incubation, absorbance was analyzed by UV-visible spectrophotometer at 640 nm.

Standard calibration curve for Gallic acid was prepared of concentration 100 to 1000  $\mu\text{g/ml}$ . The concentration (mg GAE/g) of total phenolic content was calculated by using calibration curve having linear regression  $R^2=0.936$  (Figure.2a) [21].

### **2.6.2. Estimation of total alkaloids**

About 100g of the plant materials were ground and soaked in methanol at 4°C for one day. Material was filtrated and dried by using rotary evaporator. About 2N HCl was added in filtrate. In separate funnel added 1 ml of this solution and washed three times with chloroform. About 0.1 N NaOH was added to neutralize the pH of this solution. After neutralization, 5ml of each PB (phosphate buffer) and Bromocresol green solution (BCG) was added in this mixture. 1, 2, 3 and 4 ml of chloroform was added with continuous shaking. The extracted material was shifted to 10 ml of volumetric flask and diluted with chloroform. The absorbance of the extracted mixture in chloroform was screened by UV spectrophotometer at 470 nm [22].

Standard calibration curve for Atropine was prepared of concentration 100 to 1000 $\mu\text{g/ml}$ . The concentration of total alkaloids ( $\mu\text{g/ml}$ ) were calculated by using this calibration curve having linear regression  $R^2=0.984$  (Figure.2b).

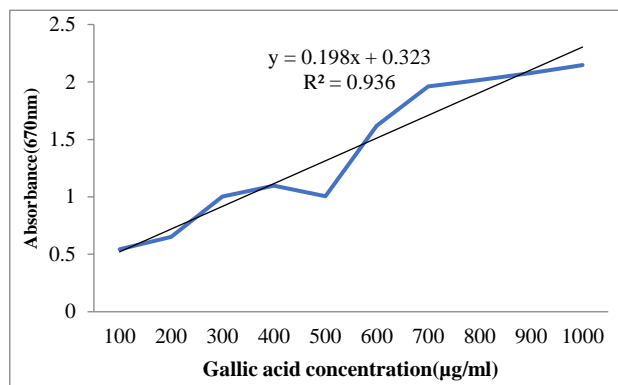


Fig. 2a

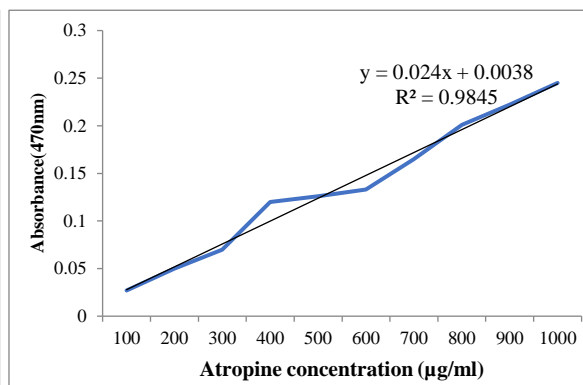


Fig. 2b

**Figure 2.** Calibration graph of (a) Gallic acid for TPC and (b) Atropine for TAC

## 2.7. Statistical analysis

The whole experiments readings were taken in triplicate and standard error was measured after getting the mean values. MANOVA was applied by using SPSS statistical software to find the significant difference followed by TUKEY test to determine the significant level of individual means.

## 3. Results

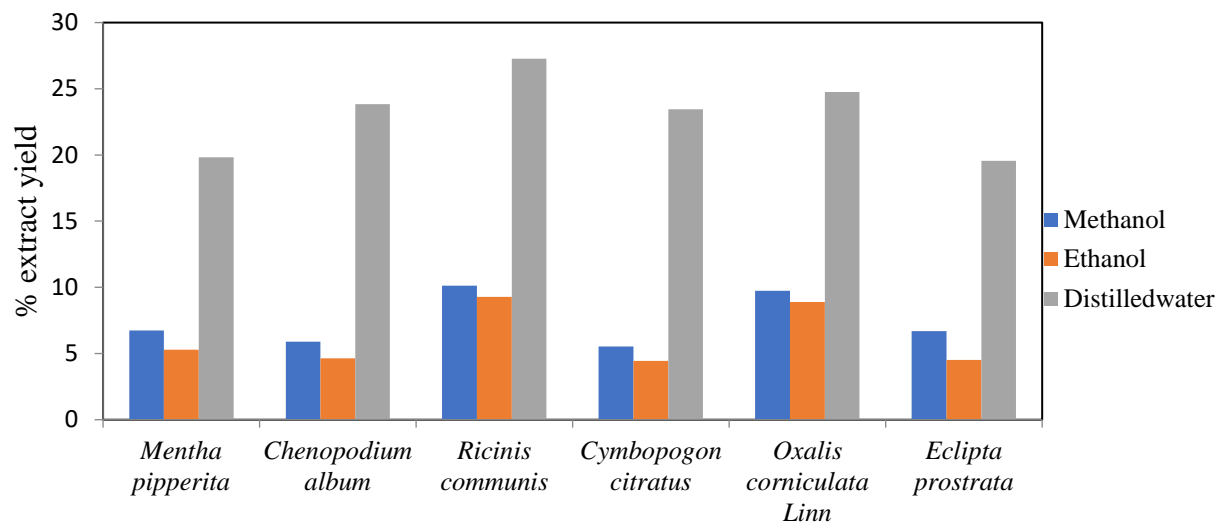
The medicinal plants become the main source of secondary metabolites. These secondary metabolites consist of phenols, alkaloids, terpenoids, tannins and saponins.

### 3.1. Quantity of Extracted material from used solvents

In screening process, phytochemicals showed different types of results in different solvents in this decreasing manner Distilled water>Methanol>Ethanol .Quantitative estimation of crude extract of studied medicinal plantsshowed decreasing trend in distilled water as *R. communis* > *O. corniculata* > *C. album* > *C. citratus* > *M. piperita* > *E. prostrata*. Quantitative estimation of crude extract of these medicinal plants studied displayed decreasing trend in Methanol *R. communis* > *O. corniculata* > *M. piperita* > *E. prostrata* > *C. album* > *C. citratus*. Quantitative estimation of crude extract of these medicinal plants studied showed decreasing trend in Ethanol *R. communis* > *O. corniculata* > *M. piperita* > *C. album* > *E. prostrata* > *C. citratus*

Quantitative results of % of crude extract of selected medicinal plants were represented in Figure 3. All the plants showed extract yield in this decreasing manner. Distilled water > Methanol > Ethanol. *R.communis* showed the highest percentage yield

in all solvents used as (27.28%) in distilled water, (10.11%) in Methanol and (9.28%) in Ethanol.



**Figure 3.** % yield of extracted material from different solvents

### 3.2. Qualitative screening the secondary metabolites of the medicinal plants

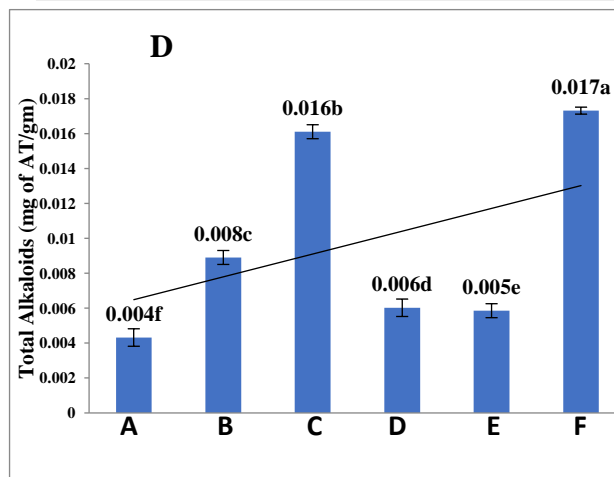
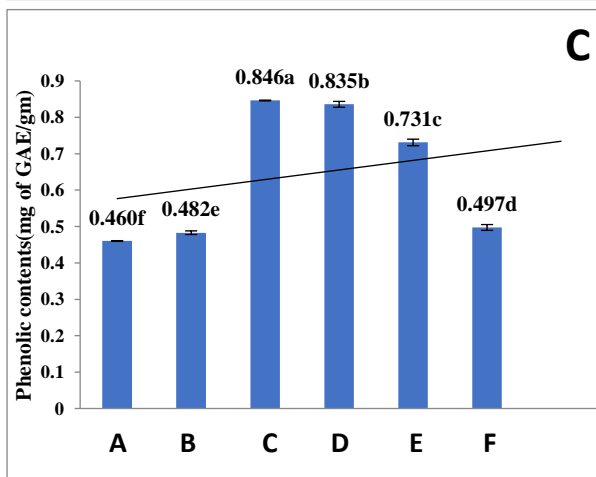
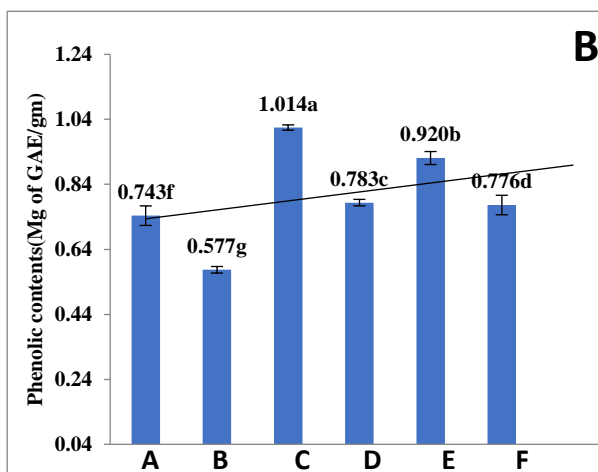
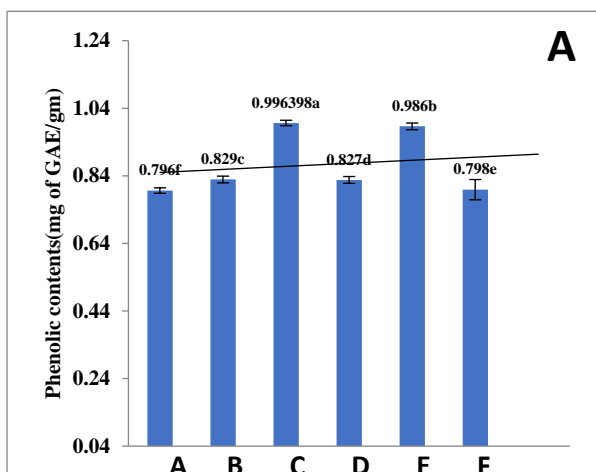
Six medicinal plants were screened for their phytochemicals characterization and results were presented in Table 2.

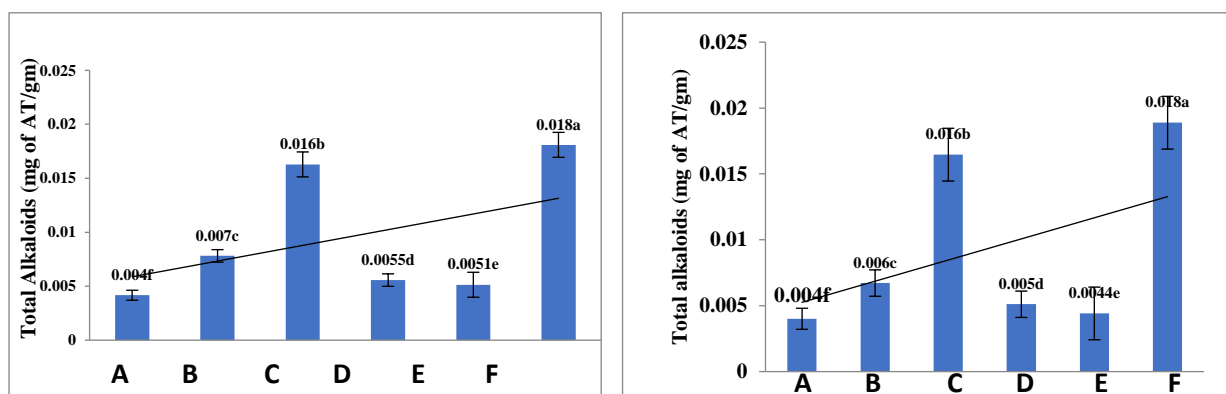
**Table 2.** Results of qualitative analysis of phytochemicals

Sr#	Phytochemicals	<i>C. album</i>	<i>R. communis</i>	<i>O. corniculata</i>	<i>M. piperita</i>	<i>C. citrates</i>	<i>E. prostrata</i>
1	Phenols	+	+	+	+	+	+
2	Alkaloids	+	+	-	+	+	+
3	Flavonoids	+	-	+	+	+	+
4	Anthocyanins	+	+	+	+	+	+
5	Tannins	+	+	-	+	+	-
6	Phlobatannins	+	-	-	+	+	+
7	Coumarins	+	-	-	+	+	+
8	Terpenoids	+	-	+	+	+	+

9	Triterpenoids	+	-	-	+	+	+
10	Steroids	+	-	+	-	+	-
11	Sterols	+	-	+	+	+	+
12	Saponins	+	+	-	+	+	+
13	Glycosides	-	+	+	-	-	+
14	Cardiac glycosides	+	-	-	-	+	-

(+) Present (-) Absent





**Figure 4.** TPC (mg/g) in (a) methanolic extract, (b) methanolic extract and (c) methanolic extract. The values of these plants represent the means of triplicate values and the error bars showed SE. Different letters present above the average bars represent the significance differences at  $p < 0.05$  (Tukey's test). A. *M. piperita* B. *C. album* C. *R. communis* D. *C. citratus* E. *O. corniculata* F. *E. prostrata*

### 3.3. Total phenolic contents (TPC) in Plant leave extract

TPC of methanolic plant extracts are given in Table 3. Descriptive statistics showed that there were significant differences existed in the phenolic contents of methanolic extract of plants Figure.4 A. The decreasing order of TPC in the medicinal plant leaves was as follows

*R. communis* > *O. corniculata* > *C. album* > *C. citratus* > *E. prostrata* > *M. piperita*

TPC of ethanolic plant extracts were shown in Table 3. Descriptive statistics showed that there were existed significant differences in the phenolic contents of ethanolic extract of plants Figure.4 B. The decreasing order of TPC in the medicinal plant leaves was as follows

*R. communis* > *O. corniculata* > *C. citratus* > *E. prostrata* > *M. piperita* > *C. album*

TPC of aqueous plant extracts showed in Table 3. Descriptive statistics showed that there were existed significant differences in the phenolic contents of aqueous extract of plants Figure.4 C. The decreasing order of TPC in the medicinal plant leaves was as follows

*R. communis* > *C. citratus* > *O. corniculata* > *E. prostrata* > *C. album* > *M. piperita*

*R. communis* showed the highest phenolic contents 1.014 mg of GAE/gm in ethanolic extract where as *M. piperita* showed lowest phenolic contents 0.460 mg of GAE/gm in distilled water. The quantity of phenolic contents in different solvents used showed the following trend *C. album* showed highest phenolic contents in methanolic extract and lowest amount in distilled water extract, *R. communis* showed highest phenolic contents in ethanolic extract and showed lowest amount in distilled water, *O. corniculata* showed



highest quantity of phenolic contents in methanolic extract and lowest in distilled water, *M. piperita* showed highest quantity of phenolic contents in methanolic extract and lowest in distilled water, *C. citratus* showed highest amount of phenolic contents in distilled water and had lowest quantity in ethanolic extract, *E. prostrate* had highest quantity of phenolic contents in methanolic extract and had lowest amount in distilled water .

**Table 3. Comparison of Total phenolic contents in different solvents (mg of GAE/gm)**

Sr#	Name of plants	Aqueous extract	Methanolic extract	Ethanolic extract
1	<i>C. album</i>	0.48±0.005	0.829±0.01	0.577±0.01
2	<i>R. communis</i>	0.846±0.001	0.996±0.008	1.014±0.008
3	<i>O. corniculata</i>	0.731±0.009	0.986±0.01	0.920±0.02
4	<i>M. piperita</i>	0.460±0.001	0.796±0.008	0.743±0.03
5	<i>C. citratus</i>	0.835±0.008	0.827±0.01	0.783±0.01
6	<i>E. prostrate</i>	0.497±0.008	0.798±0.03	0.776±0.03

#### 3.4. Total alkaloids Content in leaves of medicinal plants

The total alkaloid contents of methanolic, ethanolic and aqueous plant extracts were shown in Table. 4. Descriptive statistics showed that there were significant differences existed in total alkaloid (Figure.4 D,E, F) but the pattern remain the same which is as follows

*E. prostrata* > *R. communis* > *C. album* > *C. citratus* > *O. corniculata* > *M. piperita*

**Table 4. Comparison of alkaloids contents (mg of AT/gm) in different solvents used:**

Sr#	Name of plants	Aqueous extract	Methanolic extract	Ethanolic extract
1	<i>C. album</i>	0.006±0.001	0.008±0.001	0.007±0.001
2	<i>R. communis</i>	0.016±0.002	0.016±0.001	1.016±0.001

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3	<i>O. corniculata</i>	0.004±0.002	0.005±0.001	0.005±0.001
4	<i>M. piperita</i>	0.004±0.001	0.004±0.001	0.004±0.001
5	<i>C. citratus</i>	0.005±0.001	0.006±0.001	0.005±0.001
6	<i>E. prostrata</i>	0.018±0.002	0.017±0.001	0.018±0.001

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### 3.4. Comparison of alkaloids contents (mg of AT/gm) in different solvents used

*R. communis* showed the highest alkaloids contents 1.016 mg of AT/gm in ethanolic extract whereas *M. piperita* showed lowest quantity of alkaloids contents in each solvent used 0.004 (mg of AT/gm). Each plant showed different quantity of alkaloids contents in different solvents used which showed following trend, *C. album* showed highest alkaloids contents in methanolic extract and lowest amount in distilled water extract. *R. communis* showed highest alkaloids contents in ethanolic extract and showed lowest amount in methanolic extract. *O. corniculata* showed highest quantity of alkaloids contents in methanolic extract and lowest in distilled water. *M. piperita* showed highest quantity of alkaloids contents in methanolic extract and lowest in distilled water. *C. citratus* showed highest amount of alkaloids contents methanolic extract and has lowest quantity in distilled water. *E. prostrata* has highest quantity of alkaloids contents in distilled water and had lowest amount in methanolic extract.

### 4. Discussion

Use of medicinal plants and its traditional knowledge open up new horizons for establishing the latest drugs and combat emerging diseases. Screening of some important and active phytochemicals carried out on these plant extracts to achieve the active phytoconstituents as Phenols, Flavonoids, Glycosides, Steroids, Terpenoids, Anthocyanins, Phlobatannins, Coumarins, Tannins, Terpenoids, Triterpenoids, and Cardiac glycosides, Saponins, Sterols and Alkaloids. These traditional medicines have phytoconstituents which showed the medicinal importance, physiological activities and have the biological active ingredients which are useful as healing guide [23, 24].

All medicinal plants selected for screening were had the most of studied compounds. *C. album* most active plants as it's showed the presence of all studied phytochemicals. These phytoconstituents were comparatively studied with the workings of other peoples. Presence of alkaloids, saponins, tannins, flavonoids, glycosides, coumarins, flavonoids, steroids, sterols, cardiacglycosides, phenols and phlobatannins were recorded in the present study. These conclusion are in accordance to a prior study by Yadav and Agarwala (2011) who used diverse methods for the extraction of different parts of plant to produce a noticeable differentiation in the yield and time for extraction process and their conclusion provided

proof that aqueous and organic solvent plant extracts have medicinally important phytochemicals rub down, it justified their usage as medicines in handling of various diseases [25]. Cardiac glycosides used to treat congestive heart malfunction and cardiac arrhythmia [26]. Phenols play role in nutrient uptake, synthesis of protein, activities of enzymes, act as structural components in process of photosynthesis and allelopathy in herbs [27]. Most of the examined medicinal plants have steroidal constituents which have significant attention in medicines because of their connection with hormones such as sex hormones [28]. The usage of terpenoids in herbal medicine was also observed by pervious literature [29]. Phlobatannins reported to have mordant properties [30].

Quantitative screening of phenolics and alkaloids showed variation in different plants. There are various groups of phenols therefore it was found in high concentrations [31]. *Ricinus communis* revealed the highest amount of phenolic contents in ethanolic leaves extract than in methanolic extract and lowest amount of phenolic contents revealed in aqueous extract then the leaves of other plants. Phenolic contents have been exhibited the potent antioxidant activities [32]. Therefore there exist linear relationship between antioxidant activity and TPC. Thus phenolic having –OH groups play an important role to prevent the oxidative damage [33]. Many researchers found that phenolic contents of plants have remarkable antioxidant activities[31].

The results of present study revealed that there is great variation in the total alkaloids content of leaves in different solvents used to prepare leaves extract. Maximum alkloids content was found in the leaves of *E. prostrata* in the aqueous extract and then in ethanolic leaves extract and lowest number of alkaloids contents found in methanolic leaves extract of plants as compare to the total alkaloids contents in the leaves of same plants and from other plants. The presence of alkaloid contents played an important role in attracting the microorganism for symbiotic relation and in repelling pathogens and insects. Alkaloids have been linked with medicinal usage from long ago and their ordinary biological activity is their cytotoxicity. Literature showed that alkaloids have the analgesic and antibacterial activities. These plants which studied used to heal numerous diseases and classification and separation of the bio-active compounds developed the latest medicine of low cost which would be useful for the patients. A lot of people in urbanized countries concern customary drugs for most healthcare system [34].

## CONCLUSION

It is concluded from the results that quantity of total phenolic and alkaloids contents different in each plants at different solvents. Polar solvents give more yield as observed in our results. Phytochemical compounds are the main resource for the organization of many pharmaceutical industries. The crude drugs were identified by the present of compounds in a drug. It is concluded from these workings that *R. communis* present in highest amount in all plant extracts i.e. Distilled water, Methanol, Ethanol. It is indicated by the qualitative screening that there present a wide range of constituents in plants. The secondary

metabolites investigated to be present in various leaves are reported to have various medicinal properties. The phytochemical analysis of leaves extracts in various solvents showed the presence of secondary metabolites. The results show all the medicinal plants have phenols and anthocyanins. Alkaloids were not present in *O. corniculata* while *R. communis* had not flavonoids. Tannins were not detected in *O. corniculata* and *E. prostrata*. Phlobatannin, Coumarins and Triterpenoids were absent in *R. communis* and *O. corniculata*. Steroids were absent in *R. communis* and *M. piperita* and *E. prostrata*. Sterols were absent in *R. communis*. Saponins were absent in *O. coniculata*. Glycosides were absent in *C. album* and *M. piperita* and *C. citratus*. Cardiac glycosides were only present in *C. album* and *C. citratus*.

Thus, it was found that a wide variety of chemical constituents are present in a plant extract, each having different biological or pharmacological activities such as antioxidant, anti-cholinesterase, antimicrobial, and anti-inflammatory properties. So, in past decades, secondary metabolites derived from plant parts extraction has been found to have wonderful supply for drug progress and also applied in the fields of pharmacology and medicine.

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**Conflicts of Interest:** There are no conflicts of interest among authors to disclose.

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