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Ethno Pharmacological Screening of Selected Compounds from Crataegus Rhipidophylla Plants Grown at Swat, Pakistan

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Abstract

The present study involves the photochemical screening, mineral analysis and biological activities of *crategus rhipidophylaa*. *Crataegus rhipidophylla* collected from district Swat. It is medicinal plant. We used solvent methanol, chloroform, aqueous extract of whole plant of *crategus rhipidophylla*. The whole parts of the selected medicinal plant were washed, shade dried and then powdered for qualitative analysis. Aqueous, methanol and n-hexane extracts of whole plant were used to find out the photochemical constituents, mineral analysis, antioxidant and antibacterial activity in the *crataegus rhipidophylla.* The results of the phytochemical analysis of *crategus rhipidophylla* showed that the terpenoids, tannin, flavonoids, glycosides, alkaloids, saponins were present in all extracts except of rare phytochemicals. Mineral analysis showed that the plant extract contain copper (0.063mg/L), iron (mgL62.26/), manganese (0.448mg/L), lead (0.627mg/L), zinc (0.757mgL/), cadmium (0.211mg/L), chromium (1.332mg/L), cobalt (0.419mgL/). Antibacterail activity was carried out against five different bacterial strains: *Esheria coli, Pseudomonas Aeruoginosa, Salmonella typhae, Shigella, Staphylococcus aureus.* The antibacterial activity was determined by agar method. Antioxidant activity was carried out by DPPH method. The highest antibacterial activity; 15±0.99 mm in Hexane extract against *S. aereus* and 15±0.85 mm in methanol were obtained against *Samonellatyphae* respectively. The highest antioxidant activity was observed at 80ppm n-hexane (86.65) second highest activity was observed at 60ppm n-hexane(71.98) methanol was present at 80ppm (71.87) it was also observed that this plant is potential source of antioxidant agent and the sample has good quantity of phytochemicals. The current investigation will help the researcher as basic information for future research in manipulating the buried potential of this important plant which has not been explored so far.

1: Introduction

All around the world medicinal plants possess great influence on the health of individuals. Medicinal values of these plants are mainly based on chemical constituents they possess and their action of physiological on human beings. These biological constituents are phenolic content, alkaloids content, flavonoids and tannin content. Medicinal plants can also be used as a food plants in all over the

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world. These plants are also be used as for nursing or pregnant mothers for medicinal dedications (Adewusi, E.A. and Afolayan, A.J., 2010). From many centuries ago till now medicinal plants are utilized as a major source for medication and pharmaceutics. For synergistic neutralizing reactions there are different kinds of active compounds present in medicinal plants. The essential advantages of medicinal which are obtained from plants are comparatively harmless than synthetic, have high therapeutic benefits and high cost-effective treatment (fatima*., et al.,* 2020).

Crataegus rhipidophylla is one of the most essential medicinal plants generally found in America, Asia and Europe. In Asia including Pakistan its cultivated in the northern hilly areas of the country i.e. at high altitude regions about 1850—3000 m height. *Crataegus rhipidophylla* belong to *Rosaceae* family which are categorizes in medium sized flowering plants (Faheem *et al.,* 2020; Faheem,*et al.*,2012).

Crataegus rhipidophylla can be found as a shrub or tree and their length range from up to 7 meters (23 ft) tall. Leaf blades are dark green, with 2-4 pairs of acute or sub acuminate lobes. Its heavy thorns can be up to 1.5 cm long with white flowers while fruits are bright or dark red, 8–15 mm long and 1.3-2 times as long as wide (Donmez and Ali, 2007). Consumption of *Crataegus rhipidophylla* as a medicicinal plants have a lot of therapeutic benefits including reduced the risk of cardiovascular diseases, Central nervous system abnormality, as well as act as an anti-hepatic carcinoma and anti-cancer [Kashyap CP et al., 2012; Ju LY. 2005]. Globally *Crataegus rhipidophylla* hurbs are widely used and processed as a jam, wine, candy, vinegar, and also used in cosmetic remedies [Faheem et al., 2002]. These medicinal herbs contain vital curative ingredients including important secondary metabolites, phytochemicals, carbohydrates, flavenols, phenolics compounds, terpenoids, Choline, and amines containing compounds. *Crataegus rhipidophylla* compounds are described to have anti-oxidative potential to diminish the harmful free radicals, inhibit chronic inflammation infections, decease anxiety, anti-diabetes, prevent obesity, liver diseases, maintaining renal failure, anti-asthmatic and anti-asthmatic activities *(*Kashyap CP *et al., 2012). Crataegus Rhipodophylla* occurs naturally in Kohistan, a district in KP province of Pakistan. There are many studied reported on crataegus but current study is designed to investigate phytochemical screening, antioxidant activities and antimicrobial analysis that are not reported yet. Hence, the recent research aims to recognize the metabolite flux, chemical composition, and different levels within tissues of C. rhipidophylla and develop the chemical signatures for quality control and future scientific investigations

2: Materials and methods

2.1: Sample Collection

Fresh and young plant sample of *crataegus rhpidophyllum* (shown in Fig.1) were collected in different regions of District Kohistan, KPK Pakistan. Collection of fresh and young samples of *crataegus* rhpidophyllum was done at the same time from all regions in order to minimized environmental variations. Collected material of plant was washed with tape water for removal of dust particles and then placed in dark room for drying purpose.

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Figure 1: Plant of *crataegus rhpidophyllum*

2.2 Processing of Dried Plants

Once the plant sample dried up they were converted to fine powder with the help of juicer, motor and pistils. Plant powders were transferred to Autoclaved tight air bags for further analysis.

2.3 Preparation of plant extract

For extract preparation of plant sample four different solvents were used. 50g of plant sample were kept in 500mL of each solvent and kept in shaking incubated for 2 weeks. After storage of plant sample in solvents, rotary evaporator was used for evaporation of solvents from the plant material. Semi solid liquid was obtain after performing rotary evaporator, placed in label beaker and again store in desiccators for removal of remaining solvents. After complete evaporation of solvents different solvents extract were collected that was used for further analysis.

2.4 Phytochemical analysis

Phytochemical screening of the subject samples for the qualitative identification of tannin, saponin, terpenoids, cardiac glycosides, alkaloids, flavonoids was done by various analytical techniques in NTHRI (National tea research center, shinkiari) Pakistan (Khan *et al.*, 2011).

2.5 DPPH activity for determination of antioxidant potential

Activity of antioxidant of the plant materials were determined by using reported method of (Yen and Duh (1994**).** Stock solution of DPPH was prepared by mixing 0.015g of DPPH solution in 100mL of methanol. Working solution of DPPH was prepared by diluting with methanol until its absorbance reached to 0.98(±0.02) at 517nm on spectrophotometer. Plant sample were prepared in different dilutions (50-500µg/mL). 400µL of plant sample was mixed with 3.6mL of DPPH working solution and incubated for 30 min. After incubation absorbance of this sample was checked 517nm by using UV visible spectrophotometer. For calculation of antioxidant capacity of the plant material following formula was used:

$$\% \text{ Antioxidant Capacity} = [1 - A_s/A_c] \times 100$$

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Where A_s is the sample absorbance and A_c is the control absorbance. Ascorbic acid was used as a standard.

2.6 Antimicrobial activity of the plant sample

Antimicrobial activity of the plant material was determined by using reported method (Zain *et al.,* 2012). Agar plate was inoculated by scattering of microbial inoculum over the surface of microbes with help of sterilize cotton swab. Cork borer sterilizer was used for holes formation in each well of agar plate and sample injection was done by dropper. Specific types of antibiotics were used for validation of this activity and pasted on surface of agar well plate. After injection of sample and standard then plates were allowed to incubate for 24 hours at room temperature. Measuring scale was used to determine zone of inhibition of the sample and standard to determine.

2.7 Mineral analysis

Entirely the minerals were estimated by using the methodology of atomic absorption spectrophotometer (AAS).

3: Results and discussion

3.1: Phytochemical analysis

Comparative analysis of screening of 6 different tests was done in different fractions of *crataegus rhpidophyllum* of having aqueous, chloroform, hexane and methanol as shown in Table 1, Figure 2. After comparative analysis we revealed that screening test for flavonoids, terpenoids and glycosides were found positive in all four fractions of plant sample while other fractions showed variable results of present in one fraction while absent in other fraction. Negative results of the extract might be due to solubility in which respective compounds don't dissolve. Some research data showed that the both medicinal and edible plants possess secondary metabolites which can show different pharmacological activities. A class of flavonoids known as is flavones can be used to triggered bacterial, viral and inflammatory effects (Liu *et al.,* 2019). Previously reported data of (Alhakmani, F *et al.,* 2013) showed that these compounds can mediate antioxidant and metal chelating activity.

Table 1: Comparative analysis of different screening tests

S.No	Test	Plant sample			
		Aqueous	Chloroform	Hexane	Methanol
1	Saponins	+	-	+	-
2	Flavonoids	+	+	+	+
3	Alkaloids	-	-	-	+
4	Tannin	++	-	-	++
5	Terpenoids	+	+	+	+
6	Glycosides	+	+	+	+

Key; ++ = Highly present" + = Moderately present" - = absen

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Figure 2: Qualitative evaluation of phytochemicals compounds in various cultivars of *crataegus rhpidophyllum* plants

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3.2: Comparative analysis of antimicrobial activity

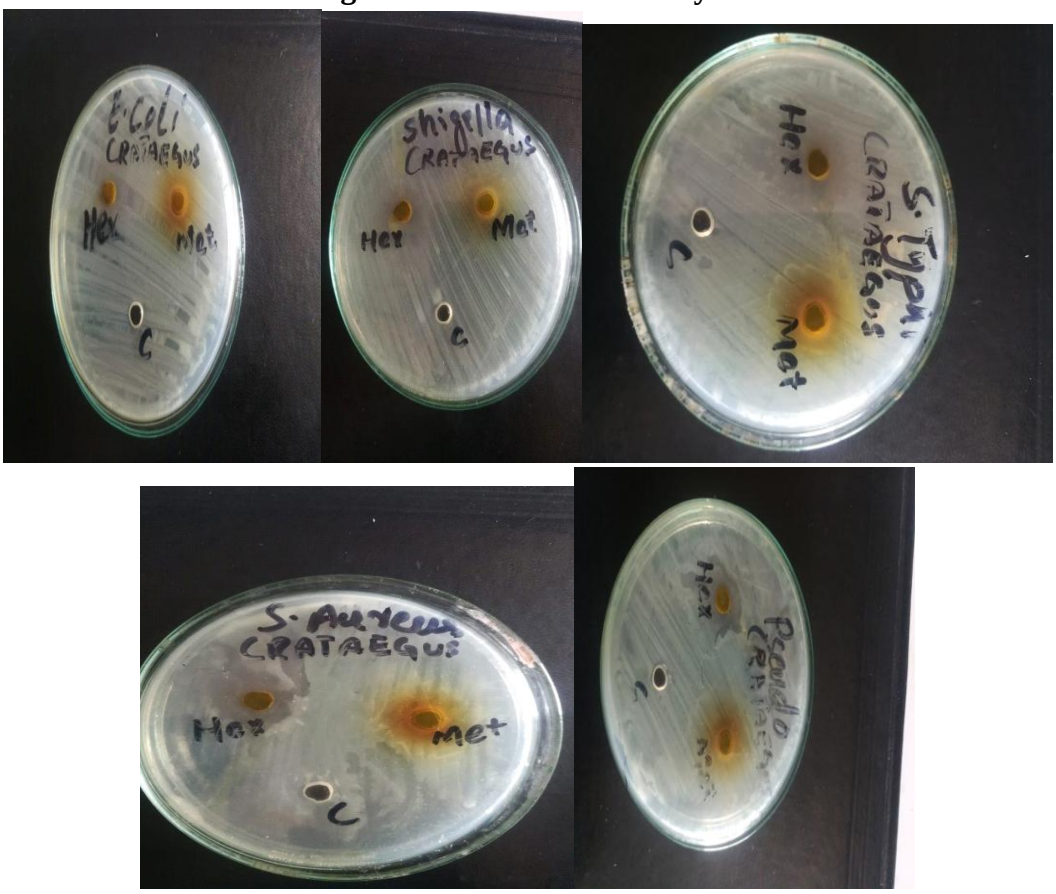
Comparative analysis of antimicrobial activity of plant sample of having two fractions of methanol and hexane showed in Table 2, Figure 3. After analysis we finally concluded that both fractions of methanol and hexane was found active against selected bacterial strains of *E.coli*, *shigilla*, *S. aereus, salmonella typhae and pseudomonas aeruginosa* except hexane that was only inactive in hexane fraction against *E.coli.* Methanol and hexane fractions were found very active against *S. aereus* and *salmonella typhae* that are shown in Table 2.

Table 2:

Comparative antimicrobial activity of plant sample

S.No	Bacterial Strains	Zone of inhibition (mm)	
		Methanol	Hexane
1	*E.coli*	13±0.89	0±0.00
2	*Shigilla*	6±0.56	11±0.96
3	*S. aereus*	12±0.71	15±0.99
4	*Samonellatyphae*	15±0.85	13±0.37
5	*Pseudomonas aeruginosa*	7±0.42	14±0.65

Figure 3: Antibacterial analysis



3.3: Mineral analysis

Analysis of mineral of plant material was determined by atomic adsorption spectrophotometer. 5 different types of mineral quantification were analyzed. After analysis it was found that all the mineral elements were found positive and exception result was found in iron quantity which was

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recorded 62mg/L in concentration and lower value of elements analysis was observed in cupper having 0.063mg/L concentration as shown in Table 3.

Table 3:Mineral analysis in plant sample

S. No	Name of Element	Concentration mg/L
1	Copper	0.063 mg/L
2	Iron	62.26 mg/L
3	Manganese	0.448 mg/L
4	Lead	0.627 mg/L
5	Zinc	0.757 mg/L
6	Cobalt	0.419 mg/L
7	Cadmium	0.211 mg/L
8	Chromium	1.332 mg/L

3.4: Antioxidant Activity by DPPH free radical scavenging assay

Four different dilutions of plant material were used to determine the antioxidant percentage inhibition of sample by DPPH method. Inhibition activity of antioxidant by plant sample was observed positive as concentrations of plant sample were increased its percentage inhibition activity was also increased that shown in Table 4.

Table 4.Antioxidant activity of plant sample

Fractions	80 PPM	60PPM	40PPM	20PPM
	DPPH	DPPH	DPPH	DPPH
Methanol	71.87±2.76	62.62±2.42	54.21±1.97	48.56±2.42
Hexane	86.65±1.98	71.98±1.91	59.94±1.84	44.21±1.97
Ascorbic Acid	89.97±1.87	77.29±1.69	66.65±1.91	59.81±2.76

CONCLUSION

After performing this research we finally conclude that *Crataegus Rhipidophylla* possess positive screening of all different test i.e., alkaloids, flavonoids, terpenoids, tannin, saponin, glycosides, having a pharmalogical and medicinal significance. The present study revealed that the studied plant is good source of iron 62.26 mg/L. It was also observed that this plant is potential source of antioxidant agents and due to its high antioxidant activity. It can also be concluded that bacterial activity showed good results in methanol and n- hexane. There is evidence that those plants which possess high concentration of phytochemical that can be used to different biological properties like

antimicrobial, anti-inflammatory and antioxidant properties. The current investigation will help the research as basic information for future research in manipulating the buried potential of this important plant which has not been explored so far.

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