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Elucidation of Phytochemicals and Antioxidant Potential of Some Selected Plant Species of Family Asteraceae

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Abstract: ***Fourteen wild plant species of family Asteraceae were selected to assess their pharmaceutical potential with the following objectives: Antioxidant properties were investigated by using three different methodologies such as DPPH free radical scavenging, Phosphomolybdenum (Total Antioxidant Activity, TAA) and FRAP assays. Quantitative phytoconstituents were examined by total phenol and total flavonoid contents. Among the selected plant species, maximum DPPH activity (%) was observed in aqua methanol and aqua acetone extract of Eclipta alba and Lagascea mollis, and FRAP in Vernonia cinerea and Ageratum houstonianum whereas TAA was in Erigeron bonariensis and Emilia sonchifolia, respectively. The Total Phenol Content (µg GAE/ mg) was highest in Erigeron bonariensis and Gnephalium pensylvanicum; while TFC (µg QE/ mg) was maximum in Xanthium strumerium and Ageratum houstonianum in aqua methanol and aqua acetone, respectively.***

Keywords: Medicinal plant, free radicals, antioxidants, phytochemicals, and Asteraceae

1. Introduction

As consequences of various metabolic and other enterprises such as smoking and pollution, various reactive oxygen species or free radicals are originated in the living system. These ROS have a single electron in their valence shell, in living systems they play important in the normal functioning by acting as communicating molecules and demolishing harmful microorganisms. Serenity between reactive oxygen species and antioxidants is necessary for all biological systems. **(Young and Woodside, 2001)**. A protective mechanism is established by the body to maintains an excellent concentration between the formation and elimination of these reactive oxygen species **(Valko *et al*., 2007; Pham-Huy *et al*., 2008)**. However, this balance is hindered by the enormous procreation of free radicals, consequences of this lead to oxidative deterioration of other cell organelles and biomolecules. These reactive oxygen species or free radicals cause various kinds of diseases, such as chronic diabetes, cardiovascular affliction, and malignancy **(Jayathilke *et al*., 2016).**

Medicinal herbs synthesized various secondary metabolites as a result of environmental stresses, theses metabolites either in the form of a pure compound or extract implemented incalculable circumstances for convenient additives and medicines for the cure of ailments and disorders. The existing literature shows that herbal medicines (especially from large families, Asteraceae, Rosaceae, Lamiaceae, etc.,) have been used from earlier times as a medicine for the cure of various ailments because members of theses families contain bioactive compounds those have pharmaceutical importance **(Hajimehdipoor *et al*., 2014).**

In the present study, fourteen different plant species of family Asteraceae were collected for evaluation of their pharmacological applications by the analysis of their antioxidant potential and biochemical profiling.

2. Material and Methods

Fourteen different wild plant species *Ageratum houstonianum, Acmella uliginosa, Blumea B. sinuata, Cirsium arvense, Eclipta alba, Erigeron bonariensis, Emilia sonchifolia, Galinsoga parviflora, Gnephalium pensylvanicum, Lagascea mollis, Sonchus asper, Sonchus olaraceus, Vernonia cinerea,* and *Xanthium Strumerium* of the family, Asteraceae were collected from Pantnagar. Identification of all the species was done with the help of eflorapantnagar, efloraindia, and Dr. D.S. Rawat, Assistant Professor, Deptt. Of Biological Sciences. Leaves of the plants were shade dried, grounded and kept for further experimentation under the laboratory conditions.

2.1 Preparation of plant extracts

The plant material (leaves) was thoroughly washed with tap water for removing dirt and then shade dried within the newspaper for approximately two weeks under the fan. The dried material was grounded by using a grinder and stored in sealed jam glass for extract preparation. Dried powder (10 g) of each plant sample was mixed in aqua-methanol and aqua-acetone (20:80 v/v) in a conical flask and sealed with paraffin wax and aluminum foil. Then for the extraction

process samples were kept in a shaker for 10 days at 150 rpm. The extracts were then filtered by using Whatman filter paper and the supernatant collected in Petri plates (labeled and weighed) and was kept undisturbed for evaporation. All extracts were then stored immediately at 4 ºC for further experimentation.

2.2 Qualitative phytoconstituent analysis

Phytochemical screening (protein, carbohydrate, tannins, quinines, flavonoids, phenols, alkaloids, saponins, terpenoids and cardiac glycosides) of all selected plant species were performed by using standardized methods proposed by **Harbone (1973)** and **Sofowara (1993).**

2.3 Assessment of antioxidant potential

The antioxidant potential of selected wild plant species of Asteraceae was evaluated in triplicates by applying 2,2'-diphenyl-1-picrylhydrazyl (DPPH) assay, Ferric reducing antioxidant power (FRAP) assay and Phosphomolybdenum assay (PMA, Total antioxidant assay).

2.3.1 2, 2'-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay

Assessment of free radical inhibition potential of plant extract was analyzed by using the method of **Brand-Williams *et al.* (1995)** with slight modifications. 0.004 % solution of DPPH was prepared freshly in dark and kept in an amber bottle at 4 ºC. The different volume of plant extract (50 to 300 µl) was added to 3 ml of DPPH solution and the total volume of the reaction mixture measure up to 4 ml with respective solvent, vortexes the reaction mixture and then incubated for 60 min in dark. The absorbance of sample mixture against blank (methanol) was taken at 517 nm in UV-vis spectrophotometer and control (negative) was also subjected to the same procedure. BHT is used as a positive control and respective solvent (80% aqua-methanol and 80% aqua-acetone) as a negative control. DPPH inhibition (%) was determined by the following equation:

$$\textbf{DPPH inhibition}(\%) = [\mathbf{1} - \left(\frac{\mathbf{At}}{\mathbf{Ac}}\right)] \times \mathbf{100}$$

Where A_t and A_c are the absorbances of sample and control at 517 nm. An increase in concentrations with a decrease in absorbance indicates higher radical scavenging capacity by the plant samples and standard.

2.3.2 Fe^{3+} reducing antioxidant power (FRAP) assay

The Fe^{3+} reducing capacity of plant samples was analyzed by using the procedure of **Benzie and Strain (1996)** with subtle modifications. Firstly a solution (fresh FRAP reagent) was prepared in 10:1:1(v/v) by mixing 300 mM acetate buffer (pH 3.6), 20 mM $FeCl_3.6H_2O$ and TPTZ respectively. An aliquot of 1 ml varying concentration (50 to 300 µl) of each methanolic extract/ acetone extract was added with 3 ml of reagent and incubated at 37 ºC for 30 min. The absorbance of the mixture was measured at 593 against blank. Trolox was used as a positive control and 80% aqua methanol and 80% aqua acetone solvent as a negative control.

2.3.3. Phosphomolybdenum assay (total antioxidant assay)

Phosphomolybdenum assay was used for determining total antioxidant activity (TAA). Determination of total antioxidant activity was performed by the reported method of **Prieto *et al.* (1999)** with minor modifications and assay is based upon the reduction rate of molybdenum (VI) to molybdenum (V) to form a green-colored complex of phosphomolybdenum. Firstly the reagent was prepared by adding (0.6 M H_2SO_4+ 4 mM ammonium molybdate + 28 mM monobasic sodium phosphate). 3 ml of freshly prepared reagent was mixed with 1 ml of different concentrations (50 to 300 µl) of each extract. The absorbance against blank was obtained at 695 nm after incubation (at 95 ºC for 90 min). For the standard reference calibration curve of ascorbic acid was used and the solvent of extraction was used for the negative control.

2.3 Quantitative phytoconstituent assays

2.3.1 Total phenol content (TPC)

Quantitative phenol content in both aqua-methanol and aqua-acetone plant extracts was analyzed via the Folin-Ciocalteu colorimetric method given by **Wolfe *et al.* (2003)** with slight modifications. Folin-Ciocalteu reagent (0.2 ml) was reacted with 0.5 ml of varying concentrations of plant extracts (50 to 300 µl) and the reaction was neutralized with 7 % saturated $NaCO_3$ (0.5 ml). The final volume of the reaction mixture was made up to 3 ml by adding distilled water. The reaction sample was vortexed and then kept 60 min for incubation, after completion of incubation time, the absorbance was measured at 765 nm against a blank. The value of absorbance was expressed in terms of gallic acid equivalent (positive control) / mg extract.

Total flavonoid content (TFC)

Flavonoid content of plant samples was analyzed by the method of **Djeridane *et al.* (2006)** with required modifications. 2 ml of plant extract was reacted with 2 ml of 2 % aluminum chloride and incubated for 60 min at room temperatures. The absorbance of the yellow-colored solution was measured at 420 nm against control (respective solvent). A quercetin calibration curve (20-

100 µg/ml) was used for the determination of flavonoid content.

3. Results and Discussion

Extraction Yield

It is much difficult to extract biologically active compounds as they are present in very less amount in all parts of the plant and variations occur in their concentration with the age, season, altitudinal differences, etc., and thus making isolation an important factor. The extraction yield and herbal extract activity depend upon the extraction method, particle size, solvent nature and polarity of various compounds in a sample, which determine the importance of applying a suitable extraction method. In the present investigation, extraction yield of fourteen different selected wild plant species of family Asteraceae was detected by applying the cold extraction method in both aqua-methanol and aqua-acetone (20: 80 v/v) and yield is shown in **Table 4.1.**

Table 1: Extraction yield (%) of different selected plant species family Asteraceae in aqua-methanol and aqua-acetone (Mean±S.E.)

S.No.	Plant Species	Yield (%)	
		Aqua-methanol	Aqua-acetone
1.	*A. houstonianum*	66.14±0.20^{i}	56.94±0.13^{i}
2.	*A. uliginosa*	10.99±0.08^{bcd}	9±0.57^{cd}
3.	*B. sinuata*	15.15±0.20^{f}	23.09±0.25^{g}
4.	*C. arvense*	13.12±0.06^{e}	9.55±0.20^{cd}
5.	*E. alba*	11.1±0.008^{bcd}	9.34±0.17^{cd}
6.	*E. bonariensis*	22.54±0.53^{h}	14.07±0.51^{e}
7.	*E. sonchifolia*	10.44±0.27^{bc}	8.8±0.08^{e}
8.	*G. parviflora*	8.1±0.65^{a}	8.05±0.53^{bc}
9.	*G. pensylvanicum*	23.98±1.37^{h}	16.05±0.53^{f}
10.	*L. mollis*	12.21±0.10^{cde}	10.56±0.17^{d}
11.	*S. asper*	20.29±0.21^{g}	28.67±0.18^{h}
12.	*S. olaraceus*	12.78±0.08^{de}	12.58±0.91^{e}
13.	*V. cinerea*	9.2±0.40^{ab}	6.7±0.97^{ab}
14.	*X. strumerium*	8±0.73^{a}	6±0.81^{a}

In the present study, the extraction yield (%) in selected wild plant species of family Asteraceae was maximum in *Ageratum houstonianum* (66.14±0.20%) and minimum in *Xanthium strumerium* (8±0.73%) of aqua-methanol and (56.94±0.13%) and (6±0.81%) in aqua-acetone again in selected both plant species respectively **(Table 4.1)**. Among the species and solvents, the yield was maximum in *A. houstonianum* may be due to the presence of low molecular weight secondary metabolites separated by the solvent methanol, which can be easily filtered out.

Qualitative Phytochemical Analysis

Recent scientific investigations have mainly limelight on the health benefits of phytochemicals. A large variety of secondary metabolites commonly present in plants labeled as phytochemicals. Most of the phytochemicals that occur in plants are assumed to be safe due to their history of consumption **(Ikram *et al.*, 2015).** The phytochemicals have gained increased scrutiny due to their pharmaceutical, therapeutic and nutritional properties and also other potential health benefits. So, there is a need to know completely about the phytochemicals pre-existing in plants to help the pharmaceutical and nutraceutical industries to elicit and take advantage of therapeutic and edible attributes of plants and serve as an alternative of natural medication against various diseases and provides a new way for drug design **(Vijayalakshmi and Ravindran, 2012).** Thus, the knowledge of plants, both qualitative and quantitative characteristics becomes necessary to mitigate the problems related to human well-being (**Khan *et al.*, 2011).**

In the present study, an attempt was made to elicit the phytoconstituents present in aqua-methanol and aqua-acetone extracts of selected plant species. Eleven different phytochemicals including alkaloids, carbohydrates, cardiac glycosides, flavonoids, phenols, proteins, saponins, tannins, steroids, terpenoids, and quinones were evaluated through standard protocols. Both aqua-methanol and aqua-acetone extracts of different wild species of family Asteraceae showed the presence of seven (proteins, carbohydrates, tannins, phenols, flavonoids, terpenoids and) out of eleven phytoconstituents (**Tables 4.2 and 4.3**).

Table 2: Qualitative phytoconstituents in aqua-methanol extracts of selected plant species

Phytochemical	Test	Plant Species													
		Ac	Ah	Bs	Ca	Ea	Eb	Es	Gp	Gpen	Lm	Sa	So	Vc	Xs
Alkaloids	Mayer's	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carbohydrate	Molisch's	++	++	+++	++	++	+++	+	++	++	++	++	++	++	++
Cardic Glycosides	Keller-Kiliani	++	+	+	+	-	-	-	+	+	-	-	-	-	-
Flavonoids	Sulfuric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	Ferric chloride	++	++	+++	+++	++	+++	++	++	+++	++	++	++	++	++
Proteins	Xanthoproteic	++	++	++	++	++	++	+	++	++	++	++	++	+	++
Quinines	Hydrochloric acid	+	++	++	+	++	++	++	++	+++	++	+	++	+	+

Saponins	Froth	-	+	++	-	+	-	-	-	+	+	+	-	-	-
Steroids	Liebermann-Burchard's	++	+	+	-	++	++	+	+	+	+	+	+	+	+
Tannins	Lead acetate	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Terpenoids	Salkowski's	+	+	-	+	+	-	+	+	+	+	+	++	+	+

Table 3: Qualitative phytoconstituents analysis in aqua-acetone extracts of selected plant species

Phytochemical	Test	Plant Species													
		Ac	*Ah*	*Bs*	*Ca*	*Ea*	*Eb*	*Es*	*Gp*	*Gpen*	*Lm*	*Sa*	*So*	*Vc*	*Xs*
Alkaloids	Mayer's	+	+	+ +	+	+	+ +	+	+	+	+	+ +	+	+++	+
Carbohydrate	Molisch's	++	++	+++	++	++	++	+	+	+	++	++	++	++	++
Cardic Glycosides	Keller-Kiliani	++	+	-	-	-	-	-	+	-	-	-	-	-	-
Flavonoids	Sulfuric acid	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Phenols	Ferric chloride	++	++	++	++	++	+	++	++	+	+	++	++	+	+
Proteins	Xanthoproteic	++	++	++	+	+	++	+	++	+	++	++	++	+	++
Quinines	Hydrochloric acid	+	++	++	+	++	++	++	++	+	++	+	++	+	+
Saponins	Froth	-	+	+	-	+	-	-	-	+	+	+	-	-	-
Steroids	Liebermann-Burchard's	++	+	+	-	+	++	+	+	+	+	+	+	+	+
Tannins	Lead acetate	+	+	+	+	+	+	+	+	+	+	+	+	+ +	+
Terpenoids	Salkowski's	+	-	-	+	+	-	+	+	-	-	+	-	+	+

3.3 Evaluation of Antioxidant potential

The normal concentration of free radicals is maintained by the self-synthesized enzymatic antioxidants such as glutathione peroxidase, superoxide dismutase, catalase and non-enzymatic antioxidants like ascorbic acid and tocopherol which protects the body from damage via free radicals. Sometimes, these antioxidants failed to remove free radicals and then antioxidants are supplied from the plant source to maintain the balance between antioxidants and free radicals **(Chand *et al.*, 2017).**

3.3.1 2, 2-diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Assay

DPPH assay is most frequently used, as it is easy and rapid in the evaluation of the antioxidant potential of the plant samples and their chemical composition. The principle behind this assay is a hydrogen donor and an electron in plant sample considered as an antioxidant has a capability of scavenging free radical (DPPH) and exhibiting color change from purple to yellow measured at 517 nm indicating the scavenging property of the plant extract or antioxidant present in plant samples **(Moon and Shibamoto, 2009).**

In the present study, the antioxidant potential of leaf extracts of both aqua-methanol and aqua-acetone in fourteen selected wild species of family Asteraceae was represented as DPPH free radical scavenging (%) by the plant extract. In aqua methanol maximum DPPH scavenging was obtained in *E. alba* **(**129.41±0.19**)** and minimum in *G. parviflora* (93±0.80) while in aqua acetone maximum *L. mollis* (117.61±0.08) and minimum in *B. sinuata* (70.73±0.85) represented by **(Fig. 1).** According to **Nisa *et al.* (2013)** in *Rumex dentatus* (92 % in butanol and 86 % in aqueous); **Sarkar *et al.* (2017)** in methanolic extract of *Centipede minima* ranging from 40.23±0.36 to 97.42±0.58 at the varying concentrations (20, 40, 60, 80 and 100 µg/ml); and **Elfalleh *et al.* (2019)** in *Stachys tmolea* (50.88±1.55 mg Trolox equivalent (TE)/g dry plant). On the other hand, **Kumar *et al.* (2013)** in *Solanum torvum* (23.0±0.8 to 50.6±0.9), *Senna auriculata* (18.2±0.8 to 55.2±0.3) showed lower values of DPPH scavenging (%). In the present study, significant differences were observed in the DPPH scavenging activity (%) in each species at varying concentrations and among different plant species ($p < 0.05$).

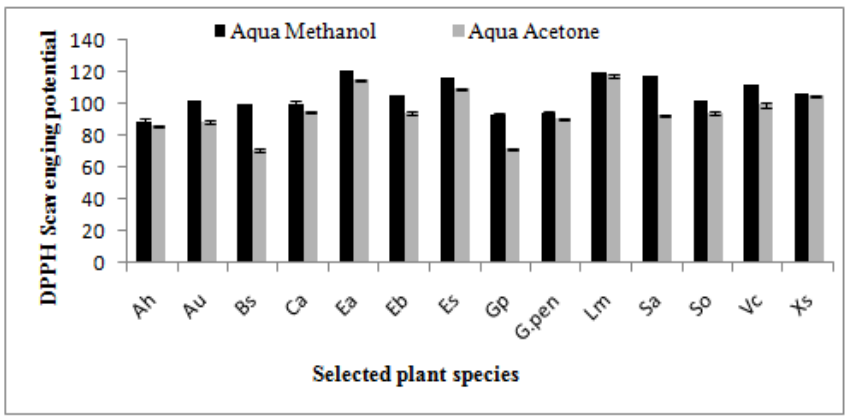

Figure 1: DPPH scavenging activity (%) in aqua-methanol and aqua-acetone leaves extracts of the selected plant species (*Ah= A. houstonianum, Au= A. uliginosa, Bs= B. sinuata, Ca= C. arvense, Ea= E. alba, Eb= E. bonariensis, Es= E. sonchifolia, Gp= G. parviflora, Gpen= G. pensylvanicum, Lm L. mollis, Sa= S. asper, So= S. olaraceus, Vc= V. cinerea,* and *Xs= X. Strumerium*).

3.3.2 Ferric reducing antioxidant power

FRAP (Fe^{3+} reducing antioxidant power) assay mainly works upon the principle, that plant sample (present antioxidants) acts as a reductant and reduces the ferric tripyridyl triazine (Fe^{3+} TPTZ) into ferrous (Fe^{2+})-TPTZ complex coupled with the intensity of color change from yellowish-green to blue, which is further evaluated by measuring the intensity of resultant colored complex at 593 nm through UV-Vis spectrophotometer **(Huang *et al.*, 2005)**. Finally, the resultant values were analyzed by a direct comparison of the sample's reducing power with standard antioxidants such as BHT equivalent (BHTE). In aqua methanol maximum ferric reducing power was obtained in *V. cinerea* (122.04±2.40) and minimum in *E. bonariensis* (54.99±2.02) and in aqua acetone maximum in *A. houstonianum* (121.78±0.13) whereas minimum in *B. sinuata* (44.42±0.03) **(Fig.2).**

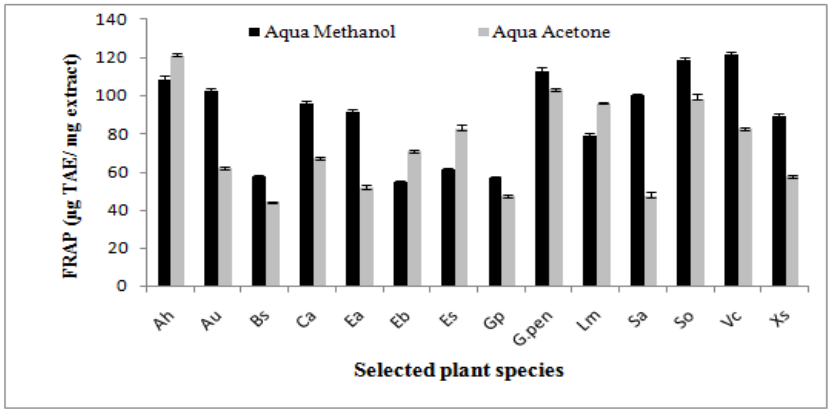

Figure 2: FRAP (µg TE/mg extract) in aqua-methanol and aqua-acetone leaves extracts of the selected plant species

3.3.3 Phospho molybdenum assay (Total antioxidant activity)

The basic principle, behind phosphomolybdenum assay, is a reduction of molybdenum (VI) to molybdenum (V) and this assay is utilized to measure the total antioxidant capacity of plant samples based on their ability to reduce the compound coupled with the color change into a green-colored complex. At 695 nm, the intensity of the green-colored product formed by the plant samples is measured by using a UV-Visible spectrophotometer and expressed directly proportional to its antioxidant capability. The data interpretation is done by the direct comparison of the sample's activity with standard antioxidant compounds, vitamin C equivalent antioxidant capacity (VEAC). In the present investigation maximum value of phosphomolybdenum or total antioxidant activity was obtained in *E. Bonariensis* (118.81±0.57) and minimum in *V. Cinerea (*42.94±0.58) in aqua methanol solvent while in aqua acetone it was highest in *E. Sonchifolia* (55.58±1.36) and lowest in *G. Parviflora* (22.57±0.26) as shown in **(Fig. 3).**

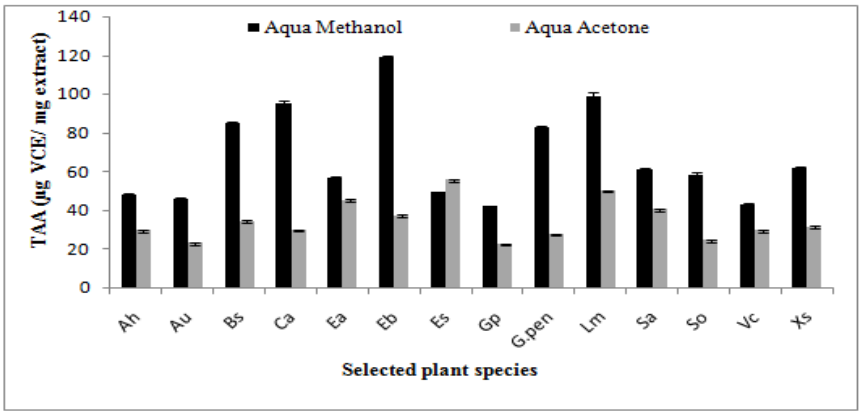

Figure 3: TAA (µg VCE/mg extract) in leaves extracts of selected plant species in aqua methanol and aqua-acetone (Mean±S.E.)

3.4 Quantitative Phytochemicals

Several phytochemicals are possessed by wild plants which are more effective in treating cough, hypersensitivity, inflammation, and can be used as a potent antioxidant when utilized through proper management strategy as they never show negative feedback mechanism as compared to the synthetic ones. So, it turns out to be essential to authenticate their quantification, safety and efficacy *in-vivo* in fresh material (**Nusrath *et al.*, 2018)**. In the present investigation, total phenol and flavonoid contents were examined in all the selected wild plant species of family Asteraceae in both aqua-methanol and aqua-acetone.

3.4.1 Total Phenol Content (TPC)

Plant phenols are an extremely diverse group of compounds due to their variability in structure and numerous substitutions for some groups. Plant phenolic composition mainly depends upon the plant species, environmental conditions, and geographical area. For some species and family, types of phenols are specific while others are ubiquitous. In recent years, phenolic compounds have gained particular attention in research and the scientific community. In the selected plant species for the present study, in aqua methanol highest phenol content was obtained in *E. bonariensis* (57.59±0.48) and lowest in *E. Sonchifolia (*29.95±0.32) whereas in aqua acetone it was maximum and minimum in *G. pensylvanicum (*45.26±0.37) and *G. parviflora*(22.59±0.37) respectively **(Fig. 4).**

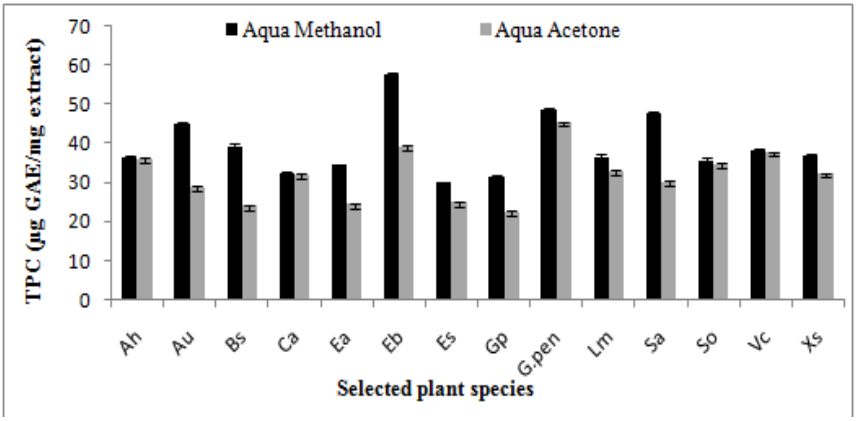

Figure 4: TPC (µg GAE/mg extract) in leaves extracts of selected plant species in aqua-methanol and aqua acetone (Mean±S.E.)

3.4.2 Total Flavonoids Content (TFC)

Flavonoids are naturally occurring most prevalent and ubiquitous classes of polyphenolic compounds formed of a 15-carbon skeleton structure having a heterocyclic and two phenyl rings present in all plant tissues. They are reported to have various biological activities, like anti-inflammatory, anti-diabetic, anti-rheumatic and anti-carcinogenic. Flavonoids have the capability of scavenging free radicals, chelating metal ions such as iron and copper and also inhibit enzymes responsible for a free radical generation **(Benavente *et al.*, 1997).** In the present study maximum flavonoids were found in *X. strumerium* (11.52±0.19) and minimum in *G. pensylvanicum* (5.11±0.05) in aqua methanol while in aqua acetone it was highest in *A. houstonianum* (13.40±0.06) and lowest in *L. mollis* (3.5±0.06).

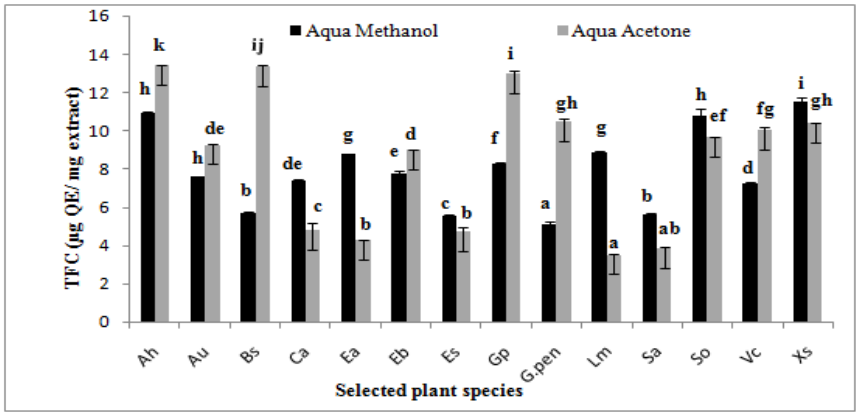

Figure 5: TFC (µg GAE/mg extract) in leaves extracts of selected plant species in aqua-methanol and aqua acetone (Mean±S.E.).

4. Conclusion and Recommendations

The findings of this investigation revealed the all selected plant species exhibited a remarkable antioxidant potential and quantity of phytochemicals. These all selected plant species are basically weeds in Pantnagar and have ethnomedicinal importance. Among selected plant species *A. houstonianum, E. alba, V.cinerea, E.bonariensis, G. pensylvanicum,* and *X. strumerium* possessed comparatively higher antioxidant along with total polyphenolic content than the rest of the plant species. Therefore, the above plant species can be a potent natural source for the preparation/development of drugs by pharmaceutical industries which are safe than the synthetic drugs. Different consolidation of the active constituents after isolation and identification can be further evaluated for a synergistic effect.

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