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Thin Layer Chromatography and Quantification of Total Contents of Phenol, Flavonoid, Tannin, Carbohydrates and Amino Acids of *Heliotropium bacciferum* Forssk Leaves and Stem Extracts

Telal M. Najeeb, Ali Elnaeim Musa and Tarig Osman Khider

College of Applied and Industrial Sciences, University of Bahri,
P.O. Box: 1606, Khartoum, Sudan

Abstract: This work aimed to identify and quantify the phytochemical components of *Heliotropium bacciferum* leaves and stem with thin layer chromatography (TLC) method in the extractions with different organic solvents namely methanol, diethyl ether and ethyl acetate. The quantitative phytochemical was showed the methanol extract had high amount of tannin (226 and 251mg/g) and high amount of polyphenol (106 and108 mg/g) and all extracts was high amount of total flavonoid (52 and 19 contented high amount of carbohydrate (4.1 and 4.0/L) follow by total amino acid was showed low content of the amino acid (1.4 and 0.79 mg) in leaves and stem respectively.

Key words: Thin Layer Chromatography · Methanol Extracts · Diethyl Ether · Ethyl Acetate · Flavonoids · Tannins · Carbohydrates · Amino Acids

INTRODUCTION

Medicinal plants represent an important component of traditional medicine in Sudan and the flora of Sudan is relatively rich in medicinal plants corresponding to the wide range of ecological habitats and vegetation zones [1-5]. These are coupled with ample inherited information in the field of medicinal plants and herbal traditional users, which originally were unique blends of indigenous cultures of various nations. Sudanese culture, although dominated by Arabic and Muslim influence, has many distinctive features. The reason for this is that Islam entered the Sudan mainly carried by leaders of suphisim. This Beings less exact form of religion it incorporated many of the pre-existing pagan beliefs and practices. Many surgical procedures are arrived out by the basir (skilled person), blood- letting and blood- cupping are commonly practiced and cautery is used to treat chronic illnesses, headache and jaundice, Al- shallag is specialized in ophthalmology. He treats cataract by removing the morbid lens in a simple operation. All forms of surgical treatment are carried out without any form of anesthesia [6].

As a continuation of previous study on *Heliotropium bacciferum* leaves and stem as source for an antibacterial and antifungal compounds [7]. The need for further investigations to support the importance of *Heliotropium bacciferum* parts especially leaves and stem.

The present research was designed to indicate amounts of biochemical constituents of *Heliotropium bacciferum* leaves and stem using the thin layer chromatography (TLC) method and determination the quantities of these components from extractions with methanol, diethyl ether and ethyl acetate.

MATERIALS AND METHODS

Collection and Identification of Plant: The leaves and stem of the *Heliotropium bacciferum* forssk were collected from soba region, southwest Khartoum (Sudan). The plant samples were identified in medicinal and aromatics plants-national research institute (National Research Center). The plant materials were dry under shade and after optimum drying, coarsely powdered.

Corresponding Author: Dr. Tarig Osman Khider, College of Applied and Industrial Sciences, University of Bahri, P.O. Box: 1606, Khartoum, Sudan.

Table 1: Solvent System and Spray reagents

Solvent System	Spray Reagent
Toluen: ethyl acetate: diethyl amine 70:20:10	UV 365nm, Dragendoff reagent
n-butanol: glacial acetic acid: water 40:10:10	Dragendoff reagent
ethyl acetate: methanol 90:10	Dragendoff reagent
Acetone: light pet rum ether: diethyl amine20:70:10	UV 365nm
Chloroform: methanol: 10% NH_3 80:40:1.5	UV 365nm
Chloroform: Acetone: diethyl amine 50:40:10	UV 365nm
Chloroform: glacial acetic acid: methanol: water 60:32:12:8	Anisaldehyde- sulphuric acid reagent/ UV 365nm
Chloroform: methanol: glacial acetic acid 47.5:47.5:5	Dragendoff / 10% $NaNO_3$
1-pronpnl:water: formic acid 90:9:1	Dragendoff / 10% $NaNO_3$
ethyl acetate: methanol: water 100:13.5:10	Iodine-potassium iodide-HCL reagent
n.Butanol: Acetic acid: Water 50:40:10	Ninhydrin

Chemicals and Reagents: Folin-Ciocalteau phenol reagent, gallic acid, anhydrous sodium carbonate, methanol, Diethyl ether, Ethyl acetate, Methanol, Quercetin, aluminium tri chloride, sodium hydroxide, Potassium ferric cyanide, Ferric chloride, Tannic acid, Leucine, Ninhydrin, Anthorn and Glucose.

Preparation of the Extracts: About 100g of *Heliotropium bacciferum* forssk were dried ground powdered leaves and stem defatted with Diethyl ether for 4 hours and extracted successively by cold method using magnetic stirring apparatus, at room temperature, the residual of the powdered plants materials were extracted again with ethyl acetate for 18 hours and finally extracted with methanol. The leaves and stem were air dried between each extraction that has involved different solvents. Each extracts were filter and concentrated under reduced pressure using the rotary evaporator.

Preparations of TLC Plates: Thin layer chromatography plates were prepared by making slurry, from 30 g of silica gel GF (Kiesel gel GF254+366), which shaken with 60 ml distilled water using 250 ml stoppered conical flask. The slurry were spray using spreader on five clean glass plates 20×20 cm and the thickness was adjusted to 0.25 mm. The plates were air dried at room temperature and activated in an oven at 105°C for one hour. The samples were dissolved in a minimum volume of their suitable solvent and applied as spots or bands at 0.5 cm from the base of the plates by glass capillary tubes. The plates were developed in appropriate different types of solvent. The basic parameter to describe migration in TLC is the R_f value [8].

$$R_f = \frac{\text{Distance moved by the solute}}{\text{Distance move by mobile phase}}$$

Determination of Total Phenol Content: Total soluble phenolics in the extract of *Heliotropium bacciferum* forsk were determined by Folin-Ciocalteu reagent according to Vermerris and Nicholson [9], with some minor modifications. Aliquots (0.1mL) of the extracts were transferred into the test tubes and their volumes were completed 4.6 mL with distilled water. 2N Folin-Ciocalteu reagent (0.3mL) and 2% Na_2CO_3 solution were vortexed and the absorbance of the mixtures as recorded after 2 hr at 750 nm, using a UV-Vis spectrophotometer, against the blank containing 0.1 mL of extraction solvent. The amount of total phenolic compounds were calculated as mg of gallic acid equivalents (GAE) from the calibration curve of gallic acid standard solution and expressed as mg gallic acid/g dry weight (DW) of the plant material. The data were presented as the average of triplicate analyses.

Determination of Total Flavonoid Content: The total flavonoid contents were determined by a colorimetric method as described by Ardestani and Yazdanparast [10]. The samples (500 µl) were mixed with 2 ml of distilled water and subsequently with 150 µl of a 15 % $NaNO_2$ solution. After 6 min, 150 µl of aluminum chloride ($AlCl_3$) solution (10%) were added and allowed to stand for 6 min. Then, 2 ml of NaOH solution (4%) were added to the mixtures. Immediately, distilled water was added to bring the final volume to 5 ml and the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was then recorded at 510 nm versus blank. All samples were duplicate, Results were expressed as mg Quercetin equivalent per gram of dry extract (mg Que/g).

Determination of Total Tannins: The tannins contents were determined by using F Cl_3 and Gelatin test [11]. A 1ml of extracts (1mg/ml) was transferring to vials, 1ml of 1% K_3 Fe $(CN)_6$ and 1ml of 1% Fe Cl_3 were added and the

volume was completed to 10ml with D.W. The color read against a reagent blank in a colorimeter at 510nm. A calibration curve was constructed using tannic acid (100- 900mg/ml) as standard and total tannins content of extraction expressed as tannic acid equivalents.

Determination of Total Carbohydrates: Total Carbohydrates method described by Mazumdar and Majumder [12]. 0.5 g of samples is hydrolyzed with 2.5 N hydrochloric acid in boiling water-bath for 2-3 hours. Then it was cooled at room temperature. Sodium carbonate ($Na_2 CO_3$) was added to it in small parts till. The extract was filtered and centrifuged. The supernatant was collected and made up to 50 ml with distilled water. A number of 1ml aliquots were taken separately into test-tubes. 4ml of 0.2% anthron reagent was added to each test-tube and then it was heated in a boiling water-bath for 10 minutes. Test-tubes were cooled at room temperature until green to dark-green coloration appeared. The Optical Density (O.D.) value of the colored solution was then measured through 630nm wavelength in a spectrophotometer at 650 nm against the blank.

Determination of Total Amino Acids: To 0.1mL of extracts was added 1mL of ninhydrin solution, make up the volume to 2mL with distilled water, heat the tube in boiling water bath for 20min. Then were added 5mL of the diluents (D.W) and mix the contents. After 15min read the intensity of the purple color against a reagent blank in a colorimeter at 570nm. The color is stable for 1h. Prepare the reagent blank as above by taking 0.1mL of 80% ethanol instead of the extract [13].

RESULTS AND DISCUSSION

Thin Layer Chromatography Heliotropium *bacciferum* Forssk leaf and stem extractsTLC profiles of the diethyl ether, ethyl acetate and methanol are presented in Tables (2, 3 and 4) The R_f values of plant extract with different solvent systems are detailed below

Table 2: R_f values for different extracts of *Heliotropium bacciferum* forces stem and leaves sprayed with Dragendroff reagent

	Developing solvent systems					
Extracts	To-EA-Dia 70:20:10	Bu-Gaa-Wa 40:10:10	EA:Me 90:10	CH-Me-Gaa 47.5:47.5:5	Po-Wa-Foa 90:9:1	Expected Metabolites
A(L)	0	0	0	0.83	0	Alkaloid
B (L)	0.56	0.75	0.73	0.87	0.81	Alkaloid
C (L)	0.38	0.73	0.73	0.75	0.68	Alkaloid
A (S)	0.54	0.73	0.75	0.87	0.81	Alkaloid
B (S)	0.44	0.73	0.75	0.87	0.75	Alkaloid
C (S)	0.53	0.75	0.75	0.75	0.81	Alkaloid

*Me = methanol, EA = ethyl acetate, To = Toluene, Dia=Diethylamide, Bu = butanol, Gaa= Glacial acetic acid= water, Po =1- pronpnol, Foa = Formic acid, CH= Chloroform.

*A (L) = diethyl ether leaf, B (L) = ethyl acetate leaf, C (L) = Methanol leaf

*A (S) = diethyl ether stem, B (S) = ehtyl acetate stem, C (S) = Methanol stem

Table 3: R_f values for different extracts of *Heliotropium bacciferum* forces stem and leaves visible with UV 365nm

	Developing solvent systems			
Extracts	AC-PE-Dia 20:70: 10	CH-AC-Dia 50:40:10	CH-Me-NH_3 80:40:1.5	Expected metabolites
A(L)	0.3,0.69,0.9	-	-	Alkaloid
B(L)	0.3, 0.9	-	-	Alkaloid
C(L)	0.9	0	0	Alkaloid
A(S)	0.3,0.9	-	-	Alkaloid
B(S)	0.3, 0.9	-	-	Alkaloid
C(S)	0.9	0.32	0.83	Alkaloid

*PE = petroleum ether, b.p 40-60°; AC= Acetone, Dia=Diethylamide, CH= Chloroform, Me= methanol.

*A (L) = diethyl ether leaf, B (L) = ethyl acetate leaf, C (L) = methanol leaf

*A (S) = diethyl ether stem, B (S) = ethyl acetate stem, C (S) = methanol stem

Table 4: R_f values for different extracts of *Heliotropium bacciferum* forces stem and leaves sprayed with Anisaldehyde and Iodine-potassium HCL reagent:

	Developing solvent systems		
Extracts	CH-Gaa-Me-Wa 60:32:12:8	EA-Me-Wa 100:13.5:10	Expected metabolites
A(L)	0.17,0.22,0.50,0.53, 0.70,0.83,0.95, 1	-	Terpenoid
B(L)	0.50, 1	-	Terpenoid
C(L)	0.33,0.42, 0.50	0.45	Terpenoid-Alkaloid
A(S)	0.50, 1	-	Terpenoid
B(S)	0.50, 1	-	Terpenoid
C(S)	0.50, 0.58	0.44	Terpenoid-Alkaloid

*EA = ethyl acetate, Gaa= Glacial acetic acid, Wa= water, CH= Chloroform, Me= methanol.

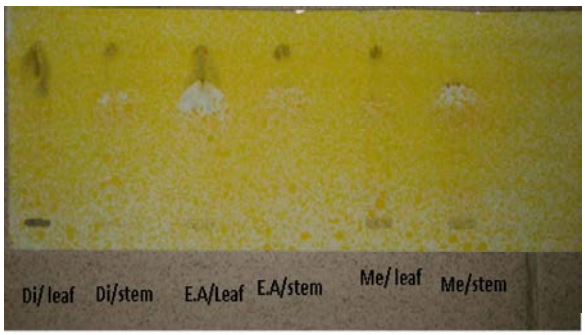
*A (L) = diethyl ether leaf, B (L) = ethyl acetate leaf, C (L) = methanol leaf

*A (S) = diethyl ether stem, B (S) = ethyl acetate stem, C (S) = methanol stem

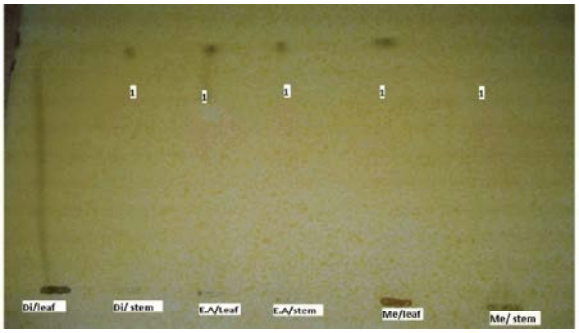
Table 5: R_f values of Methanol extracts of *Heliotropium bacciferum* forces stem and leaves sprayed with Ninhydrin

Extracts	Developing solvent systems	Expected Metabolites
	n.B-A.A- Wa 50:40:10	
C(L)	0.0	NO Amino acid
C(S)	0.39,0.51,0.63, 0.75	Amino acid

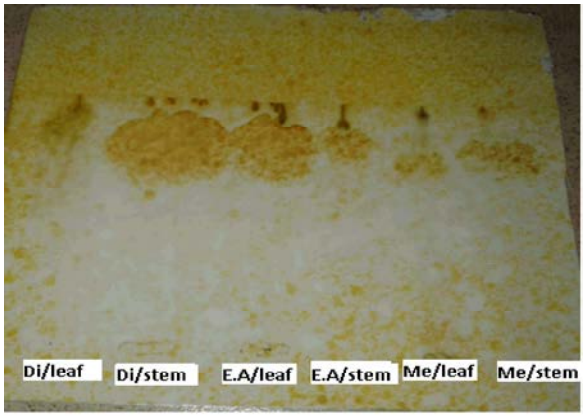
*n.B= n.butanol, A.A= acetic acid, WA= water.
* C (L) = methanol leaf
* C (S) = methanol stem



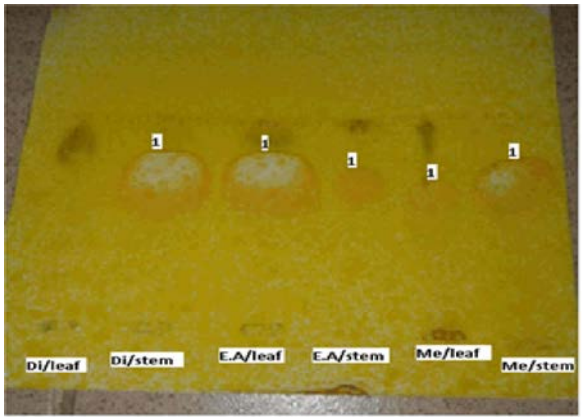
A: TLC of H. *bacciferum* leaf and stem extracts (Bu-Gaa-Wa



B: TLC of H. *bacciferum* leaf and stem extracts (EA:Me 90:10) (40:10:10)



E: TLC of H. *bacciferum* leaf and stem extracts (CH-Me-Gaa 90:10:1)

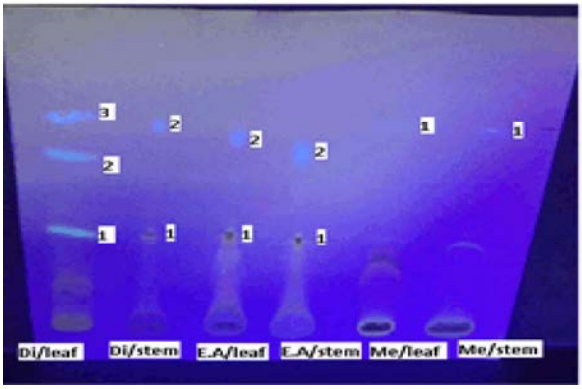


F: TLC of H. *bacciferum* leaf and stem extracts (Po-Wa- Foa 47.5:47.5:5)

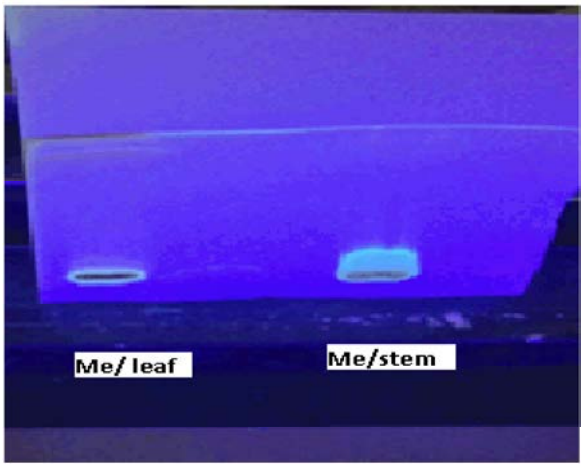
Fig. 1: TLC of plant extracts leaf and stem visualized with DRG reagent

Total Phenols: After making a standard calibration curve by gallic acid (y = 0.002260x + 0.2712, r2 =0.9951), the total phenolic content of the extract was measured. The total phenolic content calculated as mg GAE/g of dry powder. The *Heliotropium bacciferum* extracts of ethyl acetate leaf, methanol leaf and stem exhibited the highest total phenolics content. On the other hand, the total phenolics content obtained from plant extracts showed the ethyl acetate leaf and methanol leaf and stem extracts as 415.40, 406.55 and 4.15.40 mg gallic acid/100 g sample respectively, while the diethyl ether leaf and ethyl acetate stem extracts were 322.48 and 291.50 mg gallic acid/100 g sample respectively. Fig. 4.

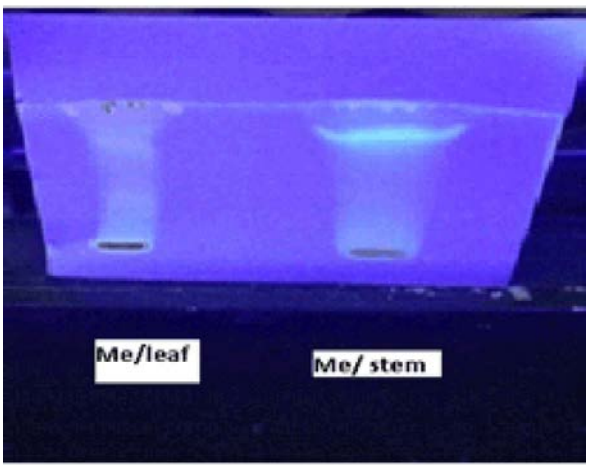
Total Flavonoid: Standard calibration curve of Quercetin was used to evaluate the content of flavonoid in the extracts (y = 0.004846x-0.03571, r2 = 0.9951). The *Heliotropium bacciferum* leaf and stem extracts of the diethyl ether contain highest flavonoid concentration followed by ethyl acetate and Methanol compared with standard Quercetin. Table 4.13. On the other hand, the total flavonids content that were obtained from plant in Diethyl ether leaf 211.66 mg Quercetin 100 g sample, Diethyl ether stem 226.11 mg Quercetin /100 g sample, ethyl acetate leaf were 178.64 mg mg Quercetin /100 g sample, ethyl acetate stem 164.20 mg Quercetin /100 g sample and



D: TLC of H. *bacciferum* leaf and stem extracts (AC-PE-Dia 20:70: 10)



H: TLC of H. *bacciferum* leaf and stem with Methanol (CH-AC-Dia50:40:10)



I: TLC of H. *bacciferum* leaf and stem with Methanol extracts Methanol extracts (CH-Me-NH_3 80:40:1.5

Fig. 2: TLC of plant extracts leaf and stem visualized with UV 365nm

Table 6: Total carbohydrate glucose standard

Glucose mg/L	0 mg/L	20 mg/L	40 mg/L	60 mg/L	80 mg/L	100 mg/L
Absorption	0	0.23	0.44	0.61	0.82	1.04
Total carbohydrate	0	23.86	44.44	61.11	81.70	103.27

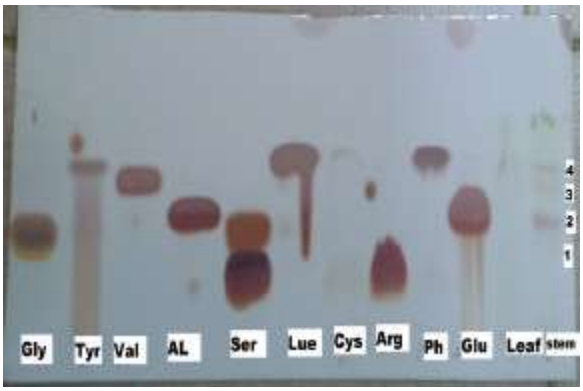
Extraction	Absorption			Average	Total Carbohydrate
Methanol leaf	8.58	9.72	10.26	9.52	934.64
Methanol stem	8.34	8.34	11.34	9.34	918.30

Methanol leaf 77.53 mg Quercetin /100 g sample and methanol stem were 172.45 Quercetin /100 g sample. Fig. 4.

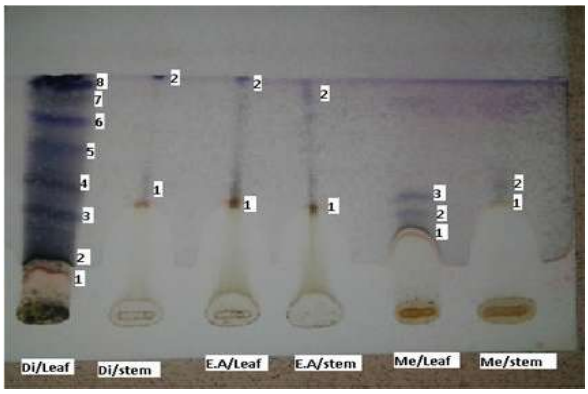
Total Tannins: Standard calibration curve of tannic acid was used to evaluate the content of tannins in the extracts (y = 0.004789x + 0.7112, r2 = 0.922). Methanol leaf extract and methanol stem extract have total tannins: 236.21 mg, 225.77 mg respectively. Fig. 4.

Total Carbohydrates: Standard calibration curve of Glucose was used to evaluate the content of carbohydrate in the methanol extracts (y = 0.0102x + 0.01333, r2 = 0.9986). Results are shown in Table 6. (mg of Glucose / liter).

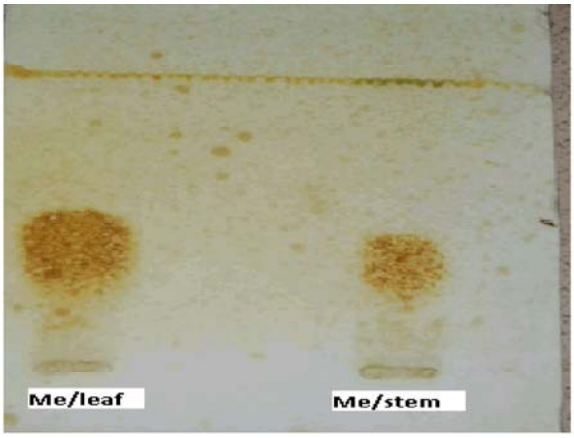
The methanol leaf and stem extracts have highest total carbohydrate 934.64 mg/L and 918.30 mg/L respectively Tables 4.18. This result showed the methanol extracts have carbohydrate concentration more than the 100 mg /L when compared with carbohydrate standard.



J: TLC of *H. bacciferum* leaf and stem extracts (n.B-A.A- WA (CH-Gaa- 50:40:10



C: TLC of *H bacciferum* leaf and stem extracts (Me-Wa 60:32:12:8)



G: TLC of *H. bacciferum* leaf and stem with Methanol extracts (EA-Me-Wa 100:13.5:10)

Fig. 3: TLC of plant extracts leaf and stem visualized with Ninhydrin reagent (**J**), with Anisaldehyde reagent (**C**) and with Iodine-potassium HCL reagent (**G**)

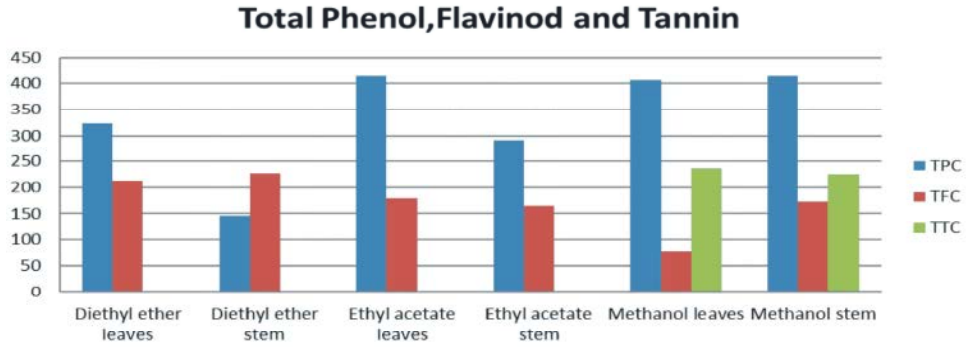


Fig. 4: Total phenol, Flavinod and Tannin Contents:

Total Amino Acid Content: Standard calibration curve of amino acid was used to evaluate the content of amino acid.

In Methanol extracts (y = 0.5255x + 0.1671, r2 = 0.09619). Results are shown in Table 7. (mg of amino Acid dry powder).

The *Heliotropium bacciferum* extracts, methanol extract leaf and stem extracts exhibited low total amino acid content compared with standard amino acid. On the other hand, the total amino acid contents of plant in methanol leaf extract 0.41 mg/ml and stem methanol extract 0.36 mg/ml. Tables 7.

Table 7: Standard amino acid leucine

Leucine mg/ml	0	0.5	0.25	0.125	0.0625	0.0313
Absorption	0	0.142	0.83	0.41	0.10	0.03
Total amino acid	0	0.59	1.90	1.10	0.51	0.38
Extraction	Absorption	Average	Total amino acid			
Methanol leaf	0.06	0.04	0.04	0.05	0.41	
Methanol stem	0.01	0.05	0.00	0.02	0.36	

CONCLUSION

The T.L.C showed that *Heliotropium bacciferum* forssk leaves and stem extracts contains different type of alkaloids, triterpenes and amino acids. The total phenol content represents the methanol extracts of leaves and stem was highest amount, followed by ethyl acetate stem and diethyl ether extracts. The total flavonoids content showed highest amount in the leaves and stem of diethyl ether followed by ethyl acetate extracts and methanol extracts. The methanol extracts of leaves and stem showed highest amount of the total tannins content. The total carbohydrate content to methanol extracts of leaves and stem showed high amount. Total amino acid content to methanol extracts leaves and stem showed low amount.

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