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Phenolic and Flavonoid contents of Grewia *tenax* polar extracts

A Project

Submitted in partial fulfillment of the requirements for the degree of Bachelors of Science (Chemistry Department)

By

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﴿ قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ﴾

سورة البقرة , الآية 31

قال العماد الاصفهاني :-

" إني رأيت انه لا يكتب انسان كتابا في يومه الا قال في غده لو غير هذا لكان أحسن ، ولو زيد كذا لكان يستحسن ، ولو قدم هذا لكان أفضل ، ولو ترك هذا لكان أجمل ، وهذا من أعظم العبر ، وهو دليل على استيلاء النقص على جملة البشر. "

الحمد لله رب العالمين

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Thank you all very much!!

Yours Sincerely:

Isra Ali Mohammed Alzwi.

Abstract

The plant *Grewia tenax* (Frosk.) Fiori. belonging to the family Tiliaceae, is a fruit producing tropical shrub or tree. It is a multipurpose plant which consumed as common household food, fodder, fuel, fiber and timber. The plant has high medicinal values and is used for treatment of numerous diseases, such as fever, diarrhea, dysentery, nausea, anemia, osteoporosis, rheumatism, bone fractures, body weakness and for bone strengthening and muscular strengthening. The secondary metabolites in the plant or crude extracts are known to be biologically active ingredients and directly responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer. In this study, quantitative spectroscopic methods coupled with coloring reagent, were used to assess the contents. Folin- Ciocalteu method was adopted to evaluate the total phenolic content of the extracts, while Aluminum chloride was used to evaluate the flavonoid content. The results showed that butanol has the highest phenolic amount with 23.0 mg GAE/g of extract, followed by chloroform with 15.2 mg GAE/g of extract, and the extracts followed the order butanol > chloroform > methanol > ethyl acetate, whereas methanol extract has the highest flavonoid contents with 3.8 mg QE/g of extract, and the extracts followed the order methanol > ethyl acetate > chloroform >butanol.

Keywords: *Grewia tenax*, total phenolic content, total flavonoid content.

ملخص البحث

نبات القضيم الذي ينتمي للعائلة (Tiliaceae) هو مثال لأنواع نباتية متعددة الأغراض والتي تعد مصدرًا للغذاء والأعلاف والألياف وحطب الوقود والأخشاب يحتوي النبات على قيم طبية عالية ويستخدم لعلاج العديد من الأمراض ، مثل الحمى والإسهال والدوسنتاريا والغثيان وفقر الدم وهشاشة العظام والروماتيزم وكسور العظام وضعف الجسم وتقوية العظام وتقوية العضلات من المعروف أن المستقبلات الثانوية في النبات أو المستخلصات الخام هي مكونات نشطة بيولوجيًا ومسؤولة بشكل مباشر عن الأنشطة المختلفة مثل مضادات الأكسدة ومضادات الميكروبات والفطريات ومضادات السرطان.

في هذه الدراسة ، استخدمت الطرق الطيفيةالكمية جانبا مع الكاشف الملون لتقييم المحتويات. اعتمدت طريقة Folin-Ciocalteu لتقييم المحتوى الكلي الفينول من المستخلصات ، في حين كلوريد الألومنيوم استخدم لتقييم محتوى الفلافونويد. وأظهرت النتائج أن بيوتانول لديها أعلى كمية الفينول بـ 23.0 مليجرام / GAE جرام للمستخلصات ، يليها الكلوروفورم بـ 15.2 مليجرام / GAEجرام من المستخلصات. واتبعت المستخلصات الترتيب الاتي بيوتانول >لكلوروفورم > الميثانول >خلات الإيثيل ، بينما احتوي مستخلص الميثانول على أعلى محتوى من الفلافونويد مع 3.8 مليجرام / QE جم من المستخلص ، واتبعت المستخلصات الترتيب الاتي الميثانول >أسيتات الإيثيل>الكلوروفورم >البيوتانول.

الكلمات المفتاحية : *Grewia tenax* ، إجمالي المحتوى الفينولي ، إجمالي محتوى الفلافونويد .

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CHAPTER 1

INTRODUCTION

1. Introduction:

G. tenax (Frosk.) Fiori is a fruit producing deciduous tropical shrub or tree, widespread in semi-arid and sub humid tropical climates. The wild shrub is the main source of the growing commercial demand for the fruit. It occurs on a large area, regenerates well and is traditionally protected during clearing and favored by farmers. Ecologically it can withstand environmental stress more easily compared with annual crops and thus make an important contribution to sustainable production without needing expensive inputs of water or fertilizer.

The shrub can give fruits three times a year, if there is sufficient rain. The plant is not only adapted to high temperatures and dry conditions, but has deep roots which stabilize sand dunes [1]. The shrub plays effectively for rehabilitation of wastelands if grown along the trees [2]

The plant has high medicinal values and is widely used for the treatment of various common diseases. *G. tenax* Fiori is reputed to cure upset of stomachs, some skin and intestinal infections, cough, fever, diarrhoea, dysentery, jaundice, rheumatism and have mild antibiotic properties. The plant preparations are used for the treatment of bone fracture and for bone strengthening. Its root and fruits are well known household remedy for the treatment of osteoporosis, tissue and wound healing. Leaves and twigs of *G.tenax* are an important component of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a

poultice to treat swelling.[3-4] *Grewia's e*xtracts are also supposed to be helpful in curing hepatitis and other such diseases.[5] The plant species has free radical scavenging activities which may be responsible for the therapeutic action against tissue damage. The plant gum was found to improve the fluidity of paracetamol granulation and could be a useful substitute binder in paracetamol tablet formulations.[6] The potential of *Grewia* gum as a film coating agent was investigated using praziquantel tablets.[7] The plant gum may serve as a good suspending agent for Ibuprofen pediatric formulation, requiring no further aid in suspension redispersibility.[8] The fruit is an important economic commodity, both locally where it is used as food and folk medicine and internationally where it has great export potential for use in food and pharmaceutical industries. It has been the subject of much global interest in research and development as it might be the solution of worldwide standing problem such as iron deficiency anemia.

Objective of the present study:

To prepare various crude extracts using different polarities of solvent and to quantitively evaluate their total phenol and flavonoids contents of G. *tenax* collected from Sebha, Libya.

CHAPTER 2

LITERATURE REVIEW

2.1 Plant profile:

2.1.1 Taxonomy :

- **Current name:** *Grewia tenax*
- **Authority:** (Forsk.) Fiori
- **Family:** Tiliaceae
- **Synonym(s) :**

-*Chadara tenax Forsk.*

-*Grewia betulifolia Juss.*

-*Gerwia chadara Lam.*

-*Gerwia erythraea schweinf.*

-*Grewia populifolia vahl.* [9]

- **Scientific name**: *Grewia tenax* (Forssk)fori.
- **Other scientific name**: *Grewia populifolia vahl.*
- **Common names:**
  - (English) : white crossberry, Phalsa cherry, Raisin bush.
  - (Arabic): gaddeim, gaddein, godem, umm ageda.
  - (others) : gangara, gangu, kanger. [9]

2.1.2 Classification :

- Kingdom : Plantae
- Division : Angiospermae

- Sub-division : Dicotyledons
- Class : Polypetalae
- Series : Thalamiflorae
- Order : Malvales
- Family : Tiliaceae
- Genus : *Grewia*
- Species : *tenax* [9]

2.2 Botanical Description :

Grewia tenax is a multistemmed small shrub up to two meters tall usually rounded but generally battered and untidy due to browsing [10]. Bark is smooth, grey, and very fibrous so that twigs are hard to break. The leaves are oval and the tip is pointed or rounded. The edge of the leave is toothed. The vein network is very clear below. Alternate, almost circular in outline, 1.5 - 4 cm in diameter [11], Margins toothed and prominently tri-nerved at the base, Stipules are conspicuous, up to 4mm. long, filiform, pubescent, falling early[10].

The young shoots and the flowers are covered with red – brown hairs. The flowers are yellow, purple of white, solitary or in twos or fours axillary placed in a terminal head about 5cm. long, the central flowers opening first, with many stamens in the center. Petals are white, about 1cm long, but usually much less, pubescent, with a linear and often 2- dentate lamina almost as

long as the sepals and narrower than the basal nectariferous claw which is circumvillous within ledged above and up to 1.5 mm long; Sepals long and recurved.[11] The fruit is orange - red at maturity, with 1- 4 spheroid lobes each rounded and fleshy about 5mm across. [12]

Figure 2.1 ***G. tenax natural growth***

Figure 2.2 ***G. tenax fruits***

2.3 Geographical Distribution and Habitat:

G. tenax is highly drought resistant and occurs in the driest savannas at desert margins and regions of higher rainfall, where it grows in thickets on termite mounds in otherwise seasonally flooded country. In the Sahel it grows in rocky places on hills and slopes, in regions with 100-600 mm of rain per annum[13]. *G. tenax* is widespread in Africa from the Transvaal and South West Africa to Ethiopia and Arabia in the North – East and through West Africa to Senegal. It is only found in the driest types of wood land or semi-desert scrub [12].

Geographically distributed in Algeria, Botswana, Chad, Djibouti, Ethiopia, Iran, Kenya, Mali, Mauritania, Morocco,

Namibia, Niger, Nigeria, Saudi Arabia, Senegal, South Africa, Sudan, Tanzania, Uganda, Zimbabwe, India, and Pakistan. [13]

Figure 2.3 Geographical Distribution of G. *tenax*

2.4 Chemical composition of *Grewia tenax* :

The chemical composition of *Grewia tenax* fruit (seeds, peel, and pulp) is shown in Table 1. The moisture, fat, fiber, and ash contents were determined using standard AOAC International methods 925.09, 932.06, 985.2912, and 923.03, respectively [14]. Based on dry weights, the moisture distribution of *Grewia tenax* fruits was as follows: seeds, 5.11 %; peel, 6.23%; and pulp, 86.52 %. The crude protein content of seeds, peel, and pulp were 7.21%, 2.12%, and 3.58 % respectively. The seeds contained more fats (10.7 %) than did the peel and pulp (1.7% and 0.2 %, respectively). The carbohydrate

content was 59.56 % for seeds, 70.74 % for peel, and 87.09 % for pulp. [14]

2.5 Nutritional properties and mineral composition :

A rich source of carbohydrates, proteins, vitamins, minerals, and constitutes which are important contributors to improving the nutritional contents of rural and urban people.

Guddaim fruit has been reported to contain large amounts of iron and so it has been used for treatment of anemia and malaria.

The fruit pulp represents only 40-50% of the whole fruit, and contains crude fiber, ash, fat, carbohydrates, iron, potassium, sulfur, phosphorus, magnesium, calcium and sodium, and a good source of amino acids (aspartic acid, threonine, serine, glutamic acid, proline glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine, histidine and arginine). [16]

Table 1. Mineral composition of G. *tenax*

Mineral content	Seeds	Peel	Pulp
Copper (Cu)	1.35±0.01a	0.78±0.003b	0.27±0.001c
Chromium (Cr)	0.02±0.002a	0.02±0.001a	0.01±0.003b
Lead (Pb)	0.01±0.005b	0.01±0.001a	0.015±0.002a
Manganese (Mn)	1.70±0.04a	0.62±0.03b	0.28±0.03c
Potassium (K)	400±0.11b	502.5±0.09a	300±0.11c
Sodium (Na)	5.82±0.03c	19.32±0.02a	11.57±0.13b
Iron (Fe)	3.65±0.07b	3.25±0.05b	4.00±0.11a

Values are means ± SDs ; values in the same row with different superscript letters differed significantly ($p \leq 0.05$) using Duncan's least significant test.

Minerals	Composition (g/100g)	mg/100g	µg/100g
Species	*Mollis* [1]	*Asiatica* [14,24]	*Asiatica* [14,25]
Potassium	NR	372	NR
Sodium	NR	17.3	NR
Calcium	NR	136	NR
Manganese	1.899±0.003	NR	NR
Zinc	0.375±0.001	NR	48
Magnesium	287.060±0.001	NR	NR
Iron	47.941±0.002	1.08	1695
Copper	0.270±0.001	NR	16
Lead	0.206±0.001	NR	NR
Country	Nigeria	USA	Pakistan
Values are expressed as mean ± SEM (n = 3).			

2.6 *Grewia* products of economic importance :

- **Food:** The fruits consumed by man and animals contain a large amount of iron and can be made into a refreshing drink. Fruit storage can be extended by drying. The dead leaves are eaten, but only while they remain on the plant. Its fruits are thirst quencher in summer season. A drink is prepared by soaking the fruit overnight, hand-pressing, sieving, and sweetening.[17]

- **Fodder:** Young leaves are consumed by livestock, they are slightly palatable at the end of dry seasons, and have fairly good feed value.[14]

- **Fuel:** The branches are used as firewood, and can be used in charcoal making. [14]

- **Fiber:** Ligno-Cellulosic Fibre with good tensile strength is made by the bark, which is used to make ropes and for binding purposes in house construction. [17]

- **Timber:** *G. tenax* wood is used in making weapons such as clubs, bows, arrows and for other general purposes. [15]

- **Poison:** A mucilaginous bark preparation is used by women against hair vermin. [17]

2.7 Medicinal importance/products:

Leaves and twigs of G. *tenax* are important components of folk medicine for the treatment of trachoma, tonsillitis, infections and are used as a poultice to treat swelling.[3-4] Because of its high iron contents, fruits of G. *tenax* are often used in special diets for pregnant women and anemic children. G. *tenax* plant is used for the treatment and prevention of iron deficiency anemia. Porridge, called Nesha, is prepared by boiling fruit pulp of G. *tenax* and millet flower given to lactating mothers. Ointment of whole plant extract applied locally for hard tissue repair and bark paste of G. *tenax* can be applied as plaster. A preparation of G. *tenax* fruit powder mixed with milk is given for the treatment of bone fracture and swelling.[18]

2.8 Previous studies of *Grewia tenax* extract:

2.8.1 Preliminary Phytochemical Screening of G. *tenax* :

Petroleum ether, chloroform and methanol extracts were prepared by subjecting powdered leaves to Soxhlet apparatus. All the extracts were subjected to preliminary phytochemical screening to detect the presence of various phytochemicals viz. alkaloids, flavonoids, tannins, glycosides, carbohydrates and saponins. [19]

Table 2. Results of preliminary phytochemical screening of extracts.

Test reagent used	Petroleum ether extract	Chloroform extract	Methanol extract	Aqueous extract
1. Alkaloids				
Mayer's reagent	-	+	-	-
Hager's reagent	-	+	-	-
Wagner's reagent	-	+	-	-
2. Carbohydrates				
Molish reagent	-	-	+	+
Fehling solution A	-	-	+	+
Fehling solution B	-	-	+	+
Benedict solution	-	-	+	+
3. Proteins				
Biuret test	-	-	-	-
Millon's test	-	-	-	-
4. Phytosterols				
Libermann– Burchard s test	+	+	-	-
Salkowski test	+	+	-	-
5. Saponins				
Foam test	-	-	+	+
6. Flavonoids				
Lead acetate test	-	-	+	+
Shinoda test	-	-	+	+
NaOH test	-	-	+	+
7. Glycosides				
Borntrager test	-	-	+	+
Keller – Killani test	-	-	+	+
Legal' s test	-	-	+	+
8.Tannins				
Ferric chloride test	-	-	+	+

CHAPTER 3

MATERIALS & METHODS

3.1 *Grewia tenax* fruit sample :

In this experiment G. *tenax* fruits were brought from local markets in Sebha, Libya. The fruits were sorted to remove low quality ones, and stored in plastic jars at room temperature until use.

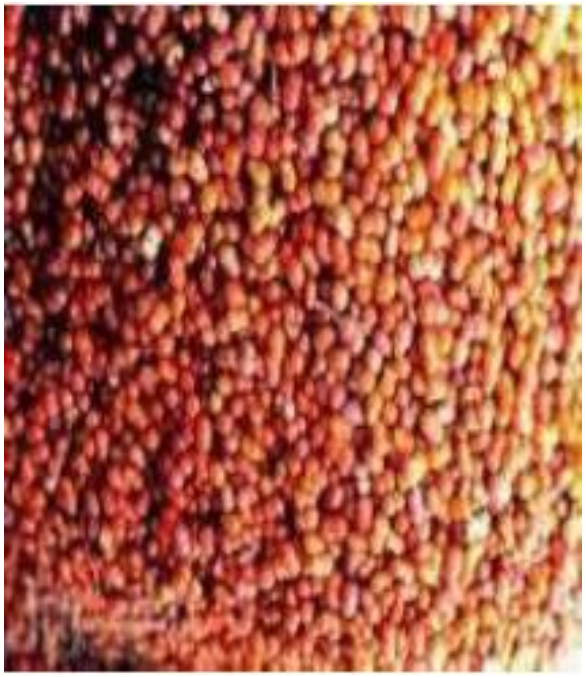


Figure 3.1 G. *tenax* fruit sample

Figure 3.2 G. *tenax* fruit sample in plastic jars

3.2 Equipment's used for extracting Flavonoids and Phenolics :

G. *tenax* fruit powder, Methanol, ethyl acetate, butanol , chloroform, condenser, mortar grinder, Thimble, RBF (round bottom flask), measuring flask, and heating mantle.

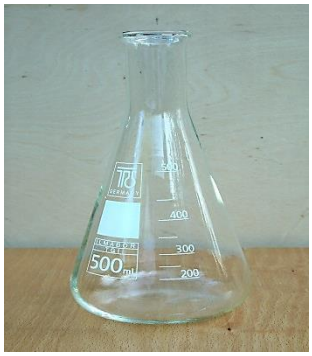


Figure 3.3 measuring flask

Figure 3.4 round bottom flask

Figure 3.5 heating mantle

Figure 3.6 mortar grinder

3.3 Sample preparation :

The fresh fruits of *G. tenax* were cleaned mechanically to take out dirt and foreign materials and the fruits were ground in a mortar grinder to small pieces, powered, and stored in closed vessel for further use.

Figure 3.7 G. *tenax* crispy fruit grinding.

3.4 Preparation of Extracts

The grinded, powered fruits (130 g) were defatted with non-polar solvents using soxhlet apparatus. The defatted plant then was extracted with methanol by shaking for 18 hours. New plant materials (130 g each) were used to prepare chloroform, ethyl acetate and butanol extracts, successively. The extracts were concentrated under reduced pressure at low temperature (40-60^{0}C). The extractive values were tabled in table 3.

Table 3. G. *tenax* extraction values

Extract	Value
Chloroform soluble	0.4g
Methanol soluble	0.2g
Ethyl acetate soluble	0.4779g
Butanol soluble	0.568g

3.5 Determination of total phenol by Folin-reagent method:

Total phenol content was determined by Folin-Ciocalteu method with modification[27]. From each crude extract (1mg) was dissolved in methanol (1mL). A total of 10% Folin-Ciocalteu reagent (10mL) with (90mL) of water in (100mL) volumetric flask. Also 5% Na_2CO_3 was prepared by dissolving (3g) of

Na_2CO_3 in water (50mL). Each crude sample (200 µL) was taken in a (10mL) plastic test tube and followed with (1.5mL) Folin-Ciocalteu reagent (10%).

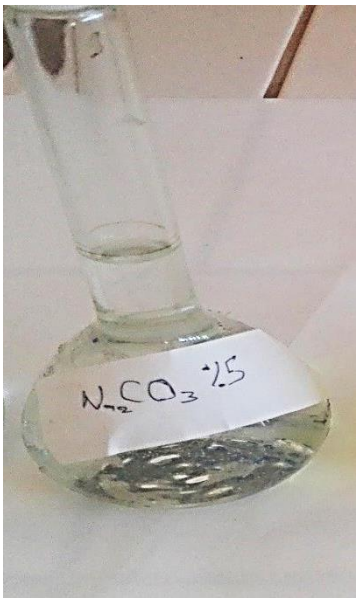


Figure 3.8 5% Na_2CO_3 in water (50mL)

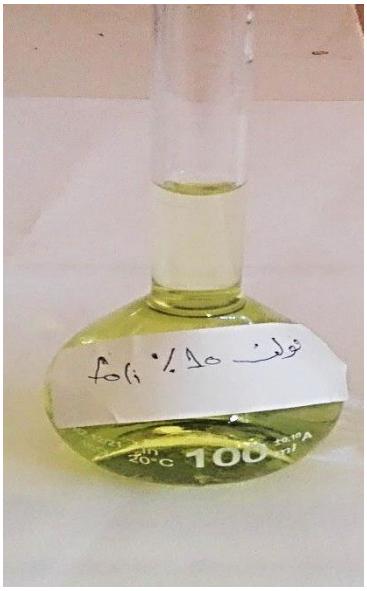


Figure 3.9 10% Folin-Ciocalteu reagent (10mL) in water (90mL)

The mixtures after shacking were kept for 5 mins. Finally, 1.5 ml of Na_2CO_3 (5%) was added to the solution and mixed well by hand. Again all the test tubes were incubated in dark. The same procedure was followed for the blank samples with replacing the Folin reagent with methanol only .

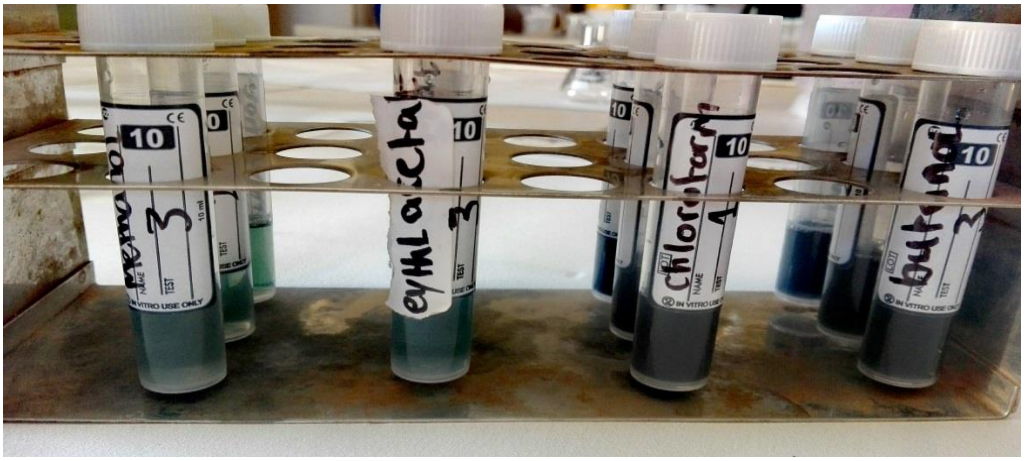


Figure 3.10 samples kept in dark for 2hrs after adding 5% Na_2CO_3 to solution.

The absorbance was measured for all the solution by using UV-spectrophotometer at constant wavelength (750 nm). All measurements were in triplicate.

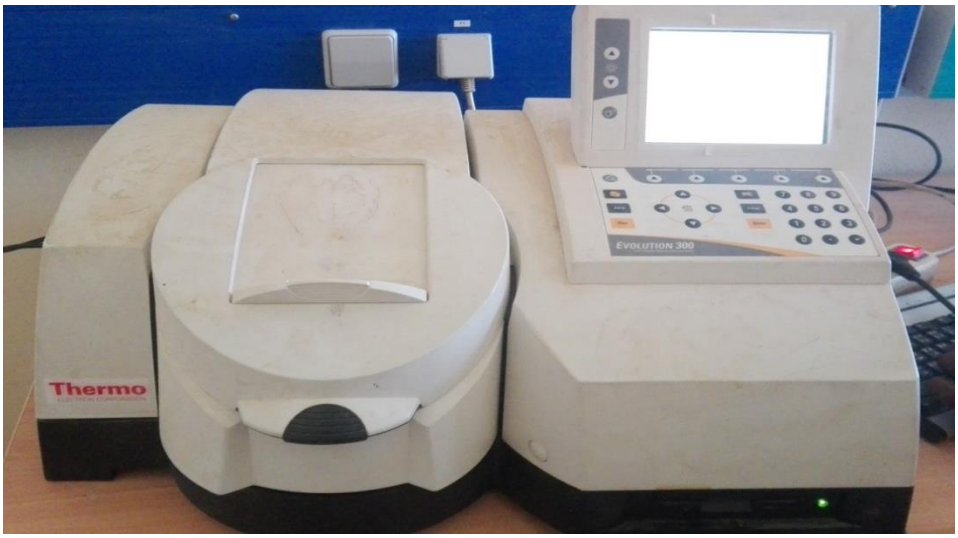


Figure 3.11 UV- spectrophotometer

3.6 Gallic acid standard curve :

Gallic acid calibration crude was prepared by Folin-Ciocalteu reagent method with modification [20]. Gallic acid (15mg) was dissolved in methanol (50mL). The concentration was of (300mg/L). This was then diluted by adding methanol to prepared serial concentrations (300, 200, 100, 80, 60, 40, 20 and 10 mg/L). This was then diluted by adding methanol to prepare serial concentrations (300, 100, 80, 60, 40, 20 and 10 mg/L). The above procedure for samples was followed for Gallic acid standard. Also all measurements were triplicate.

Figure 3.12 methanol and gallic acid

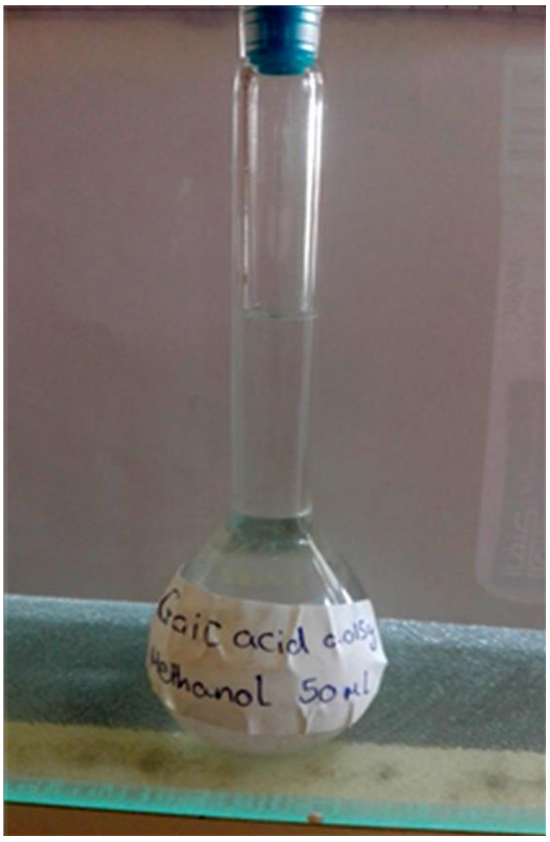


Figure 3.13 Gallic acid (15mg) dissolved in methanol (50mL)

3.7 Determination of total flavonoid content (TFC) :

Total flavonoid contents of the extracts was determined using the Dowd method as adapted by Arvouet-Grand et al.[21] Briefly, 1ml of 2% aluminum trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extracts. Absorption readings at 415 nm were taken after 10 mins against blank samples consisting of a 1 ml of extract solution with 1 ml of methanol without $AlCl_3$. All measurements were in triplicate.

Figure 3.14 1mL of 2% ($AlCl_3$) in methanol mixed in (2000 μg) extracts

Figure 3.15 Blank samples of (1mL) extract solution with (1mL) methanol without ($AlCl_3$).

3.8 Quercetin standard curve:

Quercetin standard crude was prepared by Aluminum tri chloride ($AlCl_3$) reagent method with. Quercetin (10 mg) was dissolved in methanol (25 ml). The concentration was of (300 mg/L). This was then diluted by adding methanol to prepare serial concentrations (100, 80, 60, 40, 20, 10 and 5 mg/L). The above procedure for samples was followed for Quercetin standard. Also all measurements were in triplicate.

CHAPTER 4

RESULTS & DISCUSSION

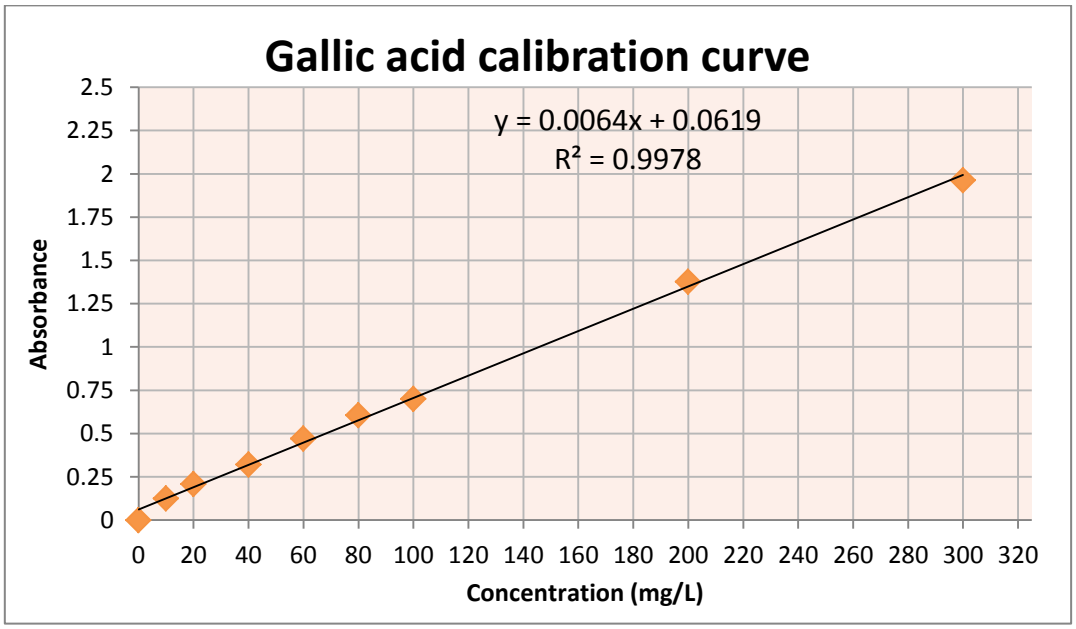
4. Results and Discussion :

4.1 Total phenolic content (TPC):

The total phenolic contents of the four crude extracts were determined by Folin- Ciocalteu method and reported in milligram Gallic Acid Equivalents per gram of extract (mg GAE/g of extract). The absorbance values, averages and standard deviations of triplicate of gallic acid standard are shown in table 4, and the Gallic acid calibration curve depicted in graph 1.

Table 4. Absorbance values, averages and standard deviations of gallic acid.

Concentration	Absorbance			Average	St. D
	1	2	3		
0	0	0	0	0	0.000
10	0.135	0.119	0.123	0.126	0.008
20	0.204	0.208	0.211	0.208	0.004
40	0.321	0.318	0.323	0.321	0.003
60	0.458	0.464	0.493	0.472	0.019
80	0.619	0.591	0.610	0.607	0.014
100	0.689	0.704	0.705	0.699	0.009
200	1.366	1.388	1.376	1.377	0.011
300	1.900	2.047	1.942	1.963	0.076



Graph 1. Gallic acid calibration curve.

The total phenolic content was calculated on the basis of the extract, using the following equation:

$$TPC = C*DF*V/P$$

Where

C: concentration of Gallic acid that equivalent to the extract in milligrams per liter.

DF: dilution factor.

V: volume of the sample used (dissolved in 10 ml)

P: weight of the extract used.

First, the absorbance readings of the different extracts were applied to the equation, obtained from calibration curve of Gallic acid (C= y – 0.0619/0.0064). This afforded the concentration of Gallic acid equivalent in mg/L of solution (**C, mg GAE/L**).

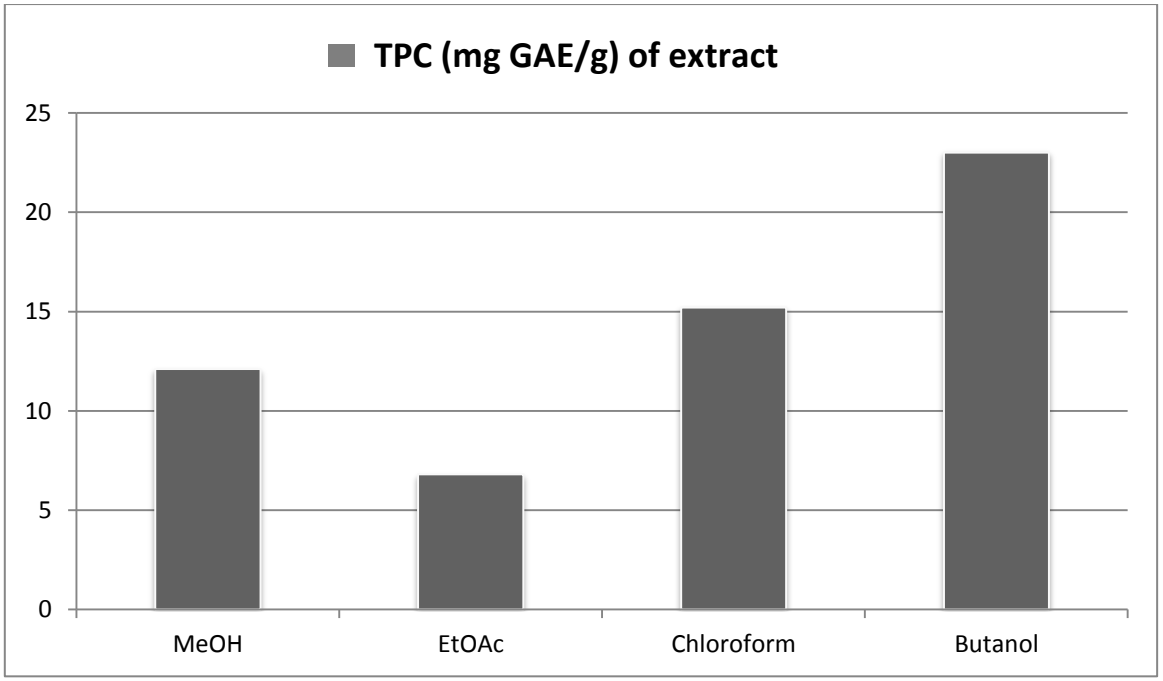
The last was used to calculate the **TPC** in **mg GAE/g of extract**. Table 8 shows the quantities of the extracts dissolved in 10 ml of methanol and the concentration of Gallic acid equivalent to the extract in mg/L . while table 6 and Graph 2 show the final results of TPC in mg GAE/g of extract, respectively.

Table 5. Quantity of the extracts used and concentrations of phenols in mg GAE/L of extract

Extract	wt. diss. In 10ml	C, mg GAE/L
MeOH	0.18	217.67
EtOAc	0.2	135.95
Chloroform	0.05	76.16
Butanol	0.1	229.60

Table 6. Total phenolic content (TPC) of different extracts.

Extract	TPC, mg GAE/g of extract
MeOH	12.1
EtOAc	6.8
Chloroform	15.2
Butanol	23.0



Graph 2. TPC, mg GAE/g of extract

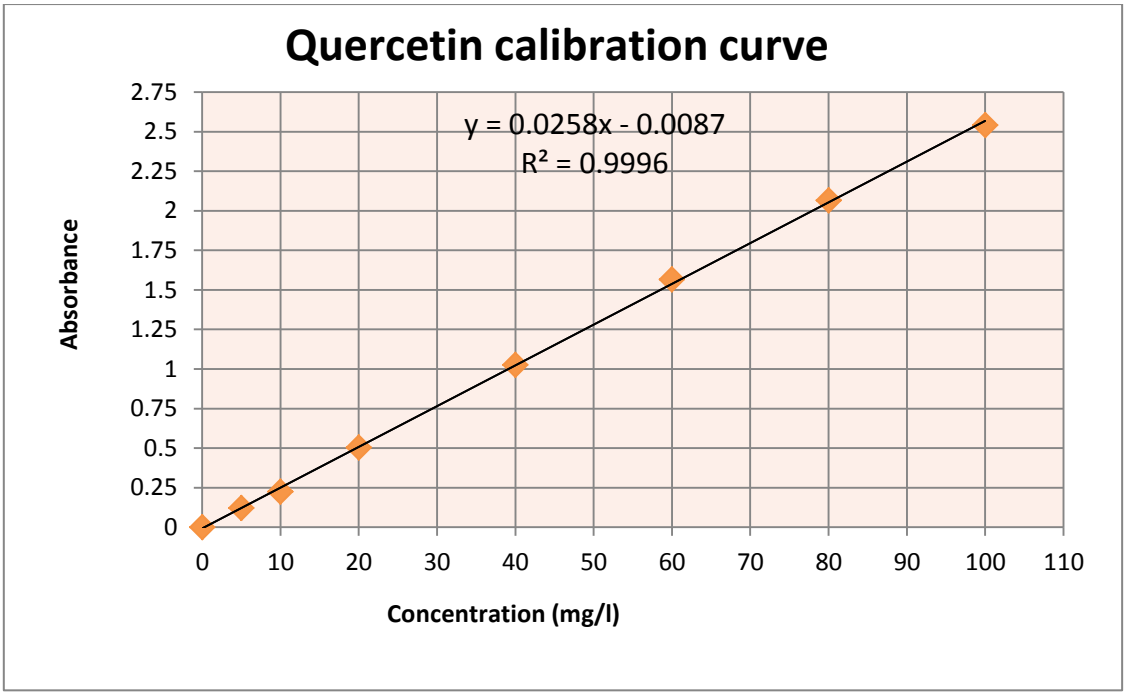
Among the four crude extracts, butanol extract showed the highest content of phenols with 23.0 mg GAE/g of extract, followed by chloroform extract with 15.2 mg GAE/g, methanol extract with 12.1 mg/g, and finally ethyl acetate with the lowest value with 6.8 mg/g. These results indicate that butanol (polar organic solvent) can extract some of these phenols. On the other hand, chloroform extracts showed higher content than methanol and ethyl acetate extracts. This is surprising, however this is may be due to presence of low polarity phenols that can be extracted by low polarity solvents.

4.2 Total flavonoid content (TFC):

The total flavonoid contents of the four crude extracts were determined using the Dowd method as adapted by Arvouet-Grand et al. and reported in milligram Quercetin Equivalents per gram of extract (mg QE/g extract). The absorbance values, averages and standard deviations of triplicates of quercetin standard solutions were shown in table 7, and the Gallic acid calibration curve depicted in graph 3.

Table 7. Absorbance values, averages and standard deviations of Quercetin

Concentration	Absorbance			Averages	St. D
	1	2	3		
0	0	0	0	0	0.000
5	0.12	0.125	0.115	0.120	0.005
10	0.239	0.177	0.257	0.224	0.042
20	0.503	0.499	0.507	0.503	0.004
40	1.013	1.029	1.036	1.026	0.012
60	1.57	1.56	1.567	1.566	0.005
80	2.066	2.066	2.063	2.065	0.002
100	2.557	2.522	2.546	2.542	0.018



Graph 3. Quercetin calibration curve

The total flavonoid content was calculated on the basis of the extract, using the following equation:

$$TFC = C*DF*V/P$$

Where

C: the concentration of Quercetin equivalent in milligrams per liter.

DF: dilution factor.

V: volume of the sample used (dissolved in 10 ml).

P: weight of the dry plant used.

First, the absorbance readings of the different extracts were applied to the equation, obtained from calibration curve of Quercetin (C= y + 0.0087/0.0258). This afforded the concentration of Quercetin equivalent in mg/L of solution (C, mg QE/L).

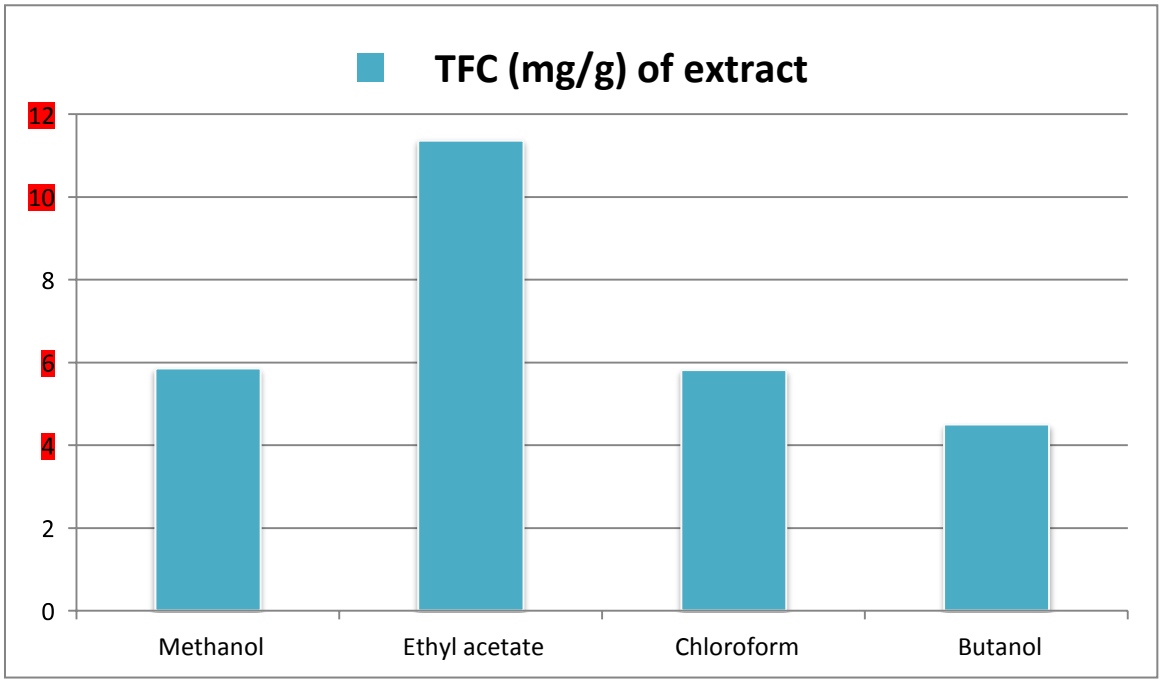
The last was used to calculate the **TFC** in **mg QE/g of extract**. Table (11) shows the quantities of the extracts dissolved in 10 ml of methanol and the concentration of Quercetin equivalent to the extract in mg/L, while table 9 and Graph 4 show the final results of TFC in mg QE/g of extract, respectively.

Table 8 . Quantity of the extracts used and concentrations of flavonoids in mg QE/L of extract

Extract	wt. diss. In 10ml	C mg QE/L
MeOH	0.18	68.41
EtOAc	0.2	61.81
Chloroform	0.2	37.79
Butanol	0.2	31.20

Table 9. Total flavonoid content (TFC) of different extracts.

Extract	TFC, mg Q/g of extract
MeOH	3.8
EtOAc	3.1
Chloroform	1.9
Butanol	1.6



Graph 4. TFC, mg QE/g of extracts

The total flavonoid contents (TFC) obtained for the four crude extracts of *G. tenax*, showed a low level of these compounds in the

extracts. This is may be due to insufficient time of extraction. Among the four crude extracts, methanol extract showed the highest content of phenols with 3.8 mg QE/g of extract, followed by ethyl acetate extract with 3.1 mg QE/g, chloroform extract with 1.9 mg/g, and finally butanol with the lowest value with 1.6 mg/g.

CHAPTER 5

CONCLUSION

5.1 Conclusion :

In the present study, the total phenol content and total flavonoid content of four crude extracts of *Grewia tenax* were studied by Folin-ciocalteu method and Aluminum chloride method, respectively. The results of total phenolic content were reported as gallic acid equivalents per gram of extract, whereas the results of total flavonoid content were reported as quercetin equivalents per gram of extract. The study showed that phenolic content of the four extracts follow the order butanol > chloroform > methanol > ethyl acetate recording 23.0, 15.2, 12.1 and 6.8 mg GAE/g of extract, respectively. The total flavonoid content of the extracts follow the order methanol > ethyl acetate > chloroform >butanol with low reading of 3.8, 3.1, 1.9 and 1.6 mg QE/g of extract, respectively.

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