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Phytochemical Composition and Antioxidant Activity of *Terminalia muelleri* and *Terminalia myriocarpa*

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Abstract

The present study was carried out to investigate the phytochemical composition and antioxidant activity of two *Terminalia* species (*T. muelleri* and *T. myriocarpa*). Antioxidant activity of the two *Terminalia* extracts was evaluated *in vitro* using DPPH, FRAP, and total phenolic content using rutin, iron sulfate, and gallic acid as reference materials. Polyphenol compounds of the two *Terminalia* leaves were investigated using HPLC and HPLC-MS/MS. It was shown that the two species leaves exhibited high antioxidant activity. The polyphenols content of *T. muelleri* and *T. myriocapa* are 0.19 and 0.155 mg equivalent of gallic acid/mg crude extract respectively. HPLC analysis of the two extracts revealed that the phenolic acids in *T. muelleri* and *T. myriocarpa* are predominantly in the free form. The main compounds are cinnamic acid and gallic acid in all analytical fractions of both *Terminalia* species. LCMS/MS analysis of the ethyl acetate extracts of the two species identified the following flavonoid compounds such as rutin, quercetin, methyl gallate, and cafoylacetylhexoside in *T. muelleri* while rutin, quercetin, epigallocatechin gallate, and myricetin were identified in *T. myriocarpa*. The antioxidant activity observed by the examined extracts may be attributed to the high amount of polyphenols. These results support the medicinal and pharmaceutical importance of *Terminalia* species.

Keywords: *Terminalia muelleri* and *Terminalia myriocarpa*, Antioxidant activity, Phenolic compounds, Flavonoids, HPLC, and LC-MS /MS.

1. Introduction:

The Combretaceae is a big family of plants consisting of approximately 20 genera and 600 species. The most popular genera are *Combretum* and *Terminalia*, both of which are widely utilized in traditional African medicine (**Ponou *et al.*, 2011**). *Terminalia* is a genus of big tree of flowering plant family *Combretaceae*, that includes about 100 species known in the tropics of the world. The name of this genus comes from a Latin word that means leaves are entirely at the tips of the shoots (**Zhang *et al.*, 2019**).

Terminalia has cyclic triterpenes and their derivatives, flavonoids, tannins, and other aromatic substances. Several previous studies reported that according to these compounds, *Terminalia* species are recognized to have several curative effects in folk medicine such as antifungal, antibacterial, anticancer, hepatoprotective, wound healing, antidiabetic, antiallergic, antiasthma, and anti-inflammatory against the acute and chronic inflammation (**Prasad *et al.*, 2004; Manna *et al.*, 2006; Ngueguim *et al.*, 2010; Chanda *et al.*, 2010; Choudhary, 2011 and Talwar *et al.*, 2011**). Therefore, it is considered vital to study the chemical composition and antioxidant activity of the two species of *Terminalia* (*T. muelleri* and *T. myriocarpa*) to prove their use in alternative remedies as nutrients and antioxidants.

2. Materials and methods:

2.1 Materials:

2.1.1 Plant material:

Fresh mature plant leaves of *Terminalia muelleri* and *T. myriocarpa* were gathered from trees growing in Giza Zoo, Egypt. Both plants

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were kindly authenticated by Dr. Treeza labib youssef, Orman garden herbarium. A voucher sample of the authenticated plant was deposited at the Department of Phytochemistry, Applied Research Center for Medicinal Plants, National Organization of Drug Control and Research (NODCAR).

2.1.2 Chemicals:

DPPH: 1, 1-diphenyl-2-picerylhydrazyl, Sigma-Aldrich Chemie, Steinheim, Germany. Folin-Ciocalteu reagent was purchased from SD fine chem. Limited, Mumbai.

All Chemicals of analytical grade and purchased from Sigma-Aldrich Laborchemikalien, GmbH, Germany. Methanol used for spectrophotometeric analysis was purchased from SD Fine Chem., Limited, Mumbai. Acetonitrile and phosphoric acid of HPLC grade (Sigma-Aldrich Laborchemikalien, GmbH, Germany).

2.1.2 Reference compounds:

Quercetin and Rutin, phenolic acids (trans–Cinnamic acid, Gallic, Chlorogenic acid, Caffeic acid, Synergic, p-Coumaric acid, and Ferulic acid) were purchased from Sigma Chemical Co., St. Louis, MO, USA.

2.2 Methods:

2.2.1 Preparation of the plant extract:

Dried and finely powdered two *Terminalia* species (*T. muelleri* and *T. myriocarpa*) were exhaustively extracted with methanol according to the method of **El-hela *et al*., 1999** for TLC and antioxidant assays.

2.2.2 Qualitative analysis by thin layer chromatography (TLC):

The methanol extract was used on a TLC plate as a spot (100 µg/ml) for chromatographic separation of the extract by the mobile phase methanol: chloroform (95:5, v/v). It was permitted to develop the chromatogram for 30 min. After completion of the chromatogram, the whole plate was sprayed with DPPH (0.15 % w/v) solution and displayed the color change from violet to yellow according to the antioxidant activity of the separated bands (**Gwatidzo *et al*., 2018**).

2.2.3 Antioxidant activity tests:

2.2.3.1 DPPH radical scavenging activity:

The free radical scavenging activity of the two examined extracts was assessed by the discoloration of the methanolic solution of DPPH according to the method of **Singh *et al*., 2011.** Rutin was used as standard**.**

2.2.3.2 Ferric reducing antioxidant power (FRAP) assay:

The determination of the FRAP assay is based on the reduction of (2,4,6-Tripyridyl-*s*-triazine) Fe^{+3} TPTZ to a blue-colored Fe^{+2} TPTZ. The method was performed according to the method of **El-Nabi1 *et al.* 2018**. Ferrous sulfate was used as standard

2.2.3.3 Spectrophotometric assay of total phenolic content:

The total phenolic content of the two examined extracts was measured using gallic acid as standard. The absorbance was recorded at 765 nm according to the method of **Meda *et al*., 2005 and Claudia *et al*., 2008.** The results were expressed as mg equivalent of gallic acid/mg of crude extract.

2.2.4 Quantitative analysis of phenolic acids by HPLC:

2.2.4.1-Sample preparation:

Dried and finely powdered two *Terminalia* species (*T. muelleri* and *T. myriocarpa*) were exhaustively extracted with methanol**.** Then methanol extract was concentrated, diluted with water, filtered and partitioned into 3 fractions. A fraction of free phenolic acids and marked (A). the other two fractions are divided into two portions. The first one was subjected to acid hydrolysis (1N HCL) and marked (B). The second one, was subjected to alkaline hydrolysis (1N KOH) and marked (C). All phenolic acids fractions (A, B, C) were evaporated at reduced pressure to form syrup like residues and these were in turn diluted in 10 ml methanol. The solutions were filtered through 0.45 µm membrane filter and injected into chromatographic column and carried out according to the method of **El-hela *et al*., 1999.**

2.2.4.2 Standard preparation:

2.2.4.2.1 Additional standard for qualitative estimation:

1ml of each fraction of both plant species (*T. muelleri* and *T. myriocarpa*) was placed in a test tube. Next, add 0.5 ml of methanolic solution of each standard of known concentration in all fractions of two plant species, with 20 µl being injected into the HPLC column.

2.2.4.2.2 External standard for quantitative estimation:

Prepare methanolic concentration of seven reference materials of cinnamic, gallic, chlorogenic, caffeic, synergic, p-Coumaric and ferulic acid with concentrations equivalent to (0.6mg/ml, 0.7mg/ml, 1.75 mg/ml, 0.15 mg/ml, 3.25 mg/ml, 0.7 mg/ml, 0.11 mg/ml), respectively. By these concentrations

of seven reference materials obtain sharp peaks, not broad peaks.

2.2.4.3 HPLC apparatus:

Ultimate 3000, Dianox, Germany. HPLC instrument equipped with pump LPG-3400 A, spectrophotometric multiwavelength detector, column hypersil PDS, RF, C18, 250 X4.5nm, 5um, and Hewlett integrator.

2.2.4.4 Elution:

Phenolic acids were isolated by isocratic elution using mobile phase (water: acetonitrile: phosphoric acid (85: 13.8: 1.2). Elution was performed at room temperature with a flow rate of 1.0 ml/min. and UV detection at 320 nm. Re-equilibration period of 10 min between individual runs.

2.2.5 Determination of flavonoids:

2.2.5.1 Determination of total flavonoids by UV spectrophotometer:

Total flavonoids in the two extracts were determined according to **Meda *et al*., 2005** using quercetin as standard. The obtained results were expressed as µg equivalent of quercetin/mg of crude extract

2.2.5.2 Qualitative and quantitative investigation of flavonoids by LC mass:

Mass quantitation is carried out using a mass spectrometer equipped with MS/MS fragmentation. MS/MS used in conjunction with quantitation is commonly coupled with a triple quadruples mass spectrometer.

2.2.5.2.1 LC mass conditions:

LC/MS, column hypersil gold C18, RF, 50 X 2.1 mm, the mass detector was Triple Stage quadruple mass spectrometer (Quantum Access Max) equipped with ion source electrospray ionization (ESI) system (Thermoscientificied, USA). Chromatographic separation was performed on reversed-phase stationary phase (C18) with injection volume 10µl, mixture of acetonitrile (A) and 0.1% formic acid in water (B) as mobile phase using gradient elution. The gradient program was set as follows: at 0.00min.90% to 10% B and at 1.0 min. 90% B. The flow rate was set at 0.4 ml/min. the column oven was kept at 40 °C and the electrospray inlet operated in a negative full-scan mode in the range of 100-1000 Dalton. The column effluent was let into MS/MS (**Chen *et al*., 2012**). These conditions were found to be suitable for the determination of the electrospray response for quercetin and rutin.

2.2.5.2.2 Extraction:

The methanolic residues of the two species *T. muelleri* and *T. myriocarpa* (3g each) were dissolved in 20 ml distilled water and partitioned between chloroform and ethyl acetate (4 x150 ml, each). The concentrated ethyl acetate fractions were dried separately to give (1.14 and 0.60 mg), respectively. The residue of each was dissolved in 10 ml methanol (HPLC grade). Filtered through a 0.45µm membrane filter (Millipore) and finally injected to LC-MS/ MS.

2.2.5.2.3 Preparation of a standard solution:

Standards of rutin and quercetin were prepared from the stock solution (0.52mg /ml, 0.54 mg /ml), respectively by dilution with methanol.

The standard concentration was prepared in range from 50 to 2500 ng/ml. The stock solutions were stored at 20 ºC. Quantification of the flavonoid compounds was achieved by measuring peak area against six concentrations of the standards, plotting standard curves, and determining the compounds in the ethyl acetate fractions.

2.2.5.2.4 Analysis of Limit of Detection (LOD) and Limit of Quantitation **(LOQ):**

The signal-to-noise ratio of 3:1 and 10:1 was used to establish LOD and LOQ, respectively.

4. Results:

4.1 Results of TLC qualitative analysis:

The development of yellowish color against the purple background on the TLC plate was observed as an indicator of the presence of antioxidant substances**.**

4.2 Results of antioxidant activity tests:

4.2.1 Results of DPPH radical scavenging activity:

Free radical scavenging activity of *T.muelleri* and *T.myriocarpa* were observed in fig.(1a-1c). Both examined extracts possess the radical scavenging activity of DPPH. This activity was augmented by elevating the concentration of the plant extracts as shown in Fig.(1a-1c). IC50 of *T. muelleri* against DPPH is 21.5 µg/ml and IC50 of *T. myriocarpa* against DPPH is 24.7 µg/ml compared to IC50 of rutin against DPPH is 16 µg/ml,

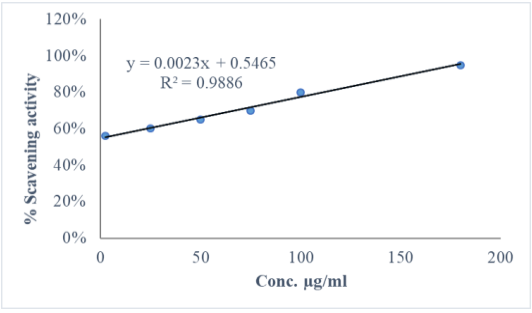


Fig.(1a): DPPH radical scavenging activity of *T. muelleri*

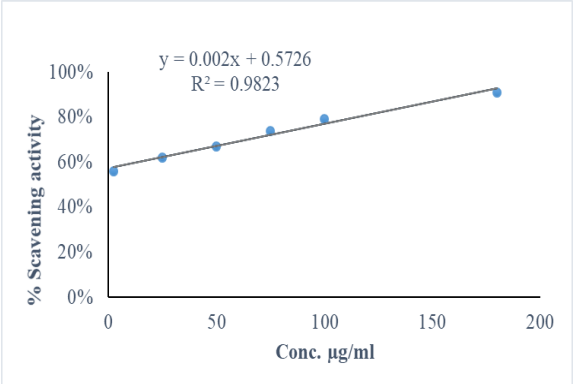


Fig.(1b): DPPH radical scavenging activity of *T. myriocarpa*

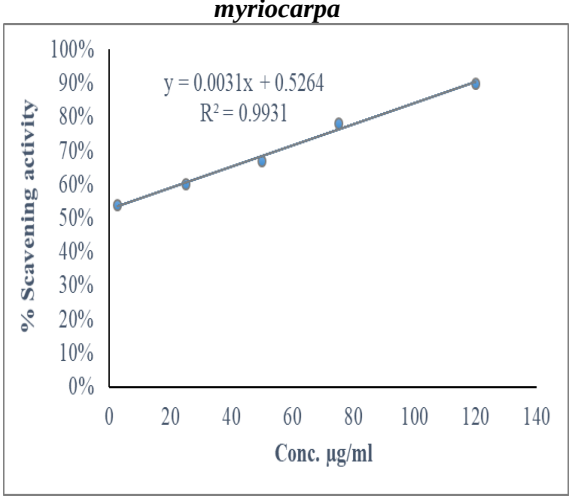


Fig. (1c): DPPH radical scavenging activity of rutin

4.2.2 Results of ferric reducing antioxidant power (FRAP):

The color of the tested extract shifts from yellow to various shades of green and blue based on the reducing power of antioxidant chemicals. A compound's reducing capacity can be a good predictor of its antioxidant ability and this activity was augmented by elevating the concentration of the plant extracts. From equation: Y= 15.888X+0.0591 Where Y=absorbance, X= corresponding concentration mg/ml as showed in Fig. (2), The antioxidant activity of *T. muelleri* and *T. myriocapa* are 0.05mg ferrous sulfate/mg crude extract and 0.027mg ferrous sulfate/mg crude extract respectively, calculated as ferrous sulfate equivalent.

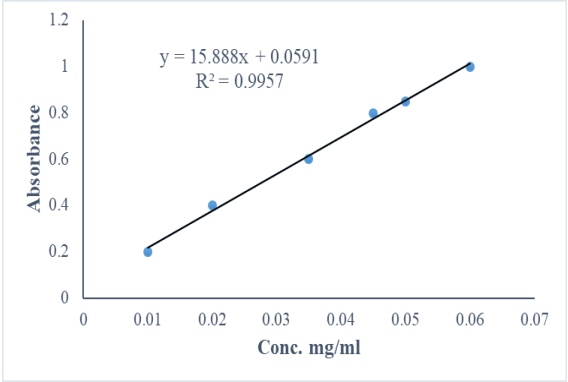


Fig. (2): Calibration curve of ferrous sulfate standard

4.2 3 Results of total phenolic content by UV spectrophotometric method:

The total polyphenols content of the 70% methanolic extract of *T. muelleri* and *T. myriocapa* were determined colorimetrically by using Folin-the Ciocalteu method. From equation: Y= 2.9231X-0.0546 Where Y=absorbance, X= corresponding concentration mg/ml. as illustrated in Fig. (3), The polyphenols content of *T. muelleri* and *T. myriocapa* are 0.19 mg gallic acid/mg crude extract and 0.155mg gallic acid/mg crude extract respectively, calculated as gallic acid equivalent.

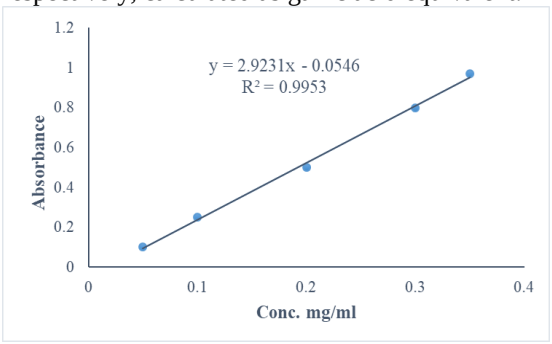


Fig. (3): Calibration curve of gallic acid standard

4.3 Results of qualitative and quantitative analysis of phenolic acids using HPLC.

The qualitative and quantitative identification of phenolic acids before and after hydrolysis in both studied species occurred by using HPLC **Tab. (1)**, **Fig. (4-16)**. The HPLC analysis of the fraction which contained free phenolic (fraction A) of *T. muelleri* and *T. myriocarpa*, revealed the presence of 12, 10 compounds, of which 4 and 5 were identified by comparing the methanolic extracts of both plants with standard phenolic acids. The quantitative analysis of the fraction (A) of *T. muelleri* showed that the main component was cinnamic acid, which comprised 77.1%, and gallic acid 21.8% of the total polyphenol(TP). The other compounds were identified as chlorogenic (0.78%of TP) and ferulic

(0.12% of TP) in small quantities. While fraction A of *T. myriocarpa* showed that the main components were cinnamic acid (68.5 % of TP), gallic acid (19.4% of TP), and synergic acids (11.7% of TP), besides the presence of the other compounds as chlorogenic (0.19 % of TP) and ferulic acids (0.14% of TP) were identified in small quantities. Meanwhile, there were eight and five unknown peaks in primary extracts of both *T. muelleri* and *T. myriocarpa* respectively. HPLC analysis of the fraction obtained through acid hydrolysis (fraction B) of *T. muelleri* and *T. myriocarpa* showed the presence of 8 and 10 peaks respectively. In parallel with the free phenolic acid fraction of both species, only cinnamic and gallic acid were identified. Although the following compounds: caffeic, synergic, and P-coumaric acids were identified in fraction B of *T. muelleri*, and the P-coumaric was identified in *T. myriocarpa.* These compounds were not present in the primary extract (A) of both species but were identified in their acid hydrolysis extracts, it may be presumed that it was originally a glycoside that decomposed during hydrolysis into secondary compounds. The fraction B of *T. muelleri* and *T. myriocarpa* contained 3 and 7 compounds, which could not be identified. The cinnamic (64.7%, 77.1% of TP) and gallic acids (33.3%, 22 % of TP) were the major components of fraction B *of T. muelleri and T. myriocarpa respectively*. The qualitative analysis of the alkaline hydrolysis fraction of *T. muelleri* (fraction C) revealed eight chemicals, three of which were recognized as cinnamic, gallic, P-coumaric, and ferulic acids, and five of which could not be identified. Whereas, the quantitative analysis of alkaline hydrolysis of *T. myriocarpa* revealed 14 compounds, seven of which were identified as cinnamic, gallic, chlorogenic, caffeic, synergic, P-Coumaric, and ferulic acids, and seven of which were not. The Caffeic acid and/or P-coumaric acid were proved to be present in the alkaline fraction of *T. muelleri* and *T. myriocarpa* compared with their corresponding primary fractions of both species.

Their presence in alkaline fractions indicated that they are derivatives of original esters.

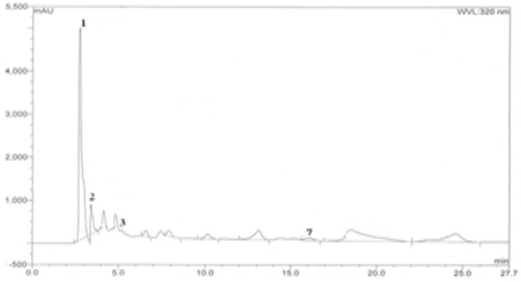


Fig. (4): HPLC chromatogram of alcoholic extract of *T. muelleri*

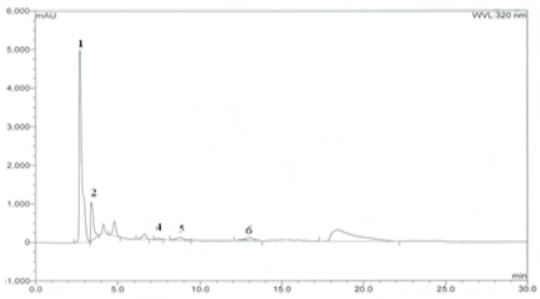


Fig. (5): HPLC chromatogram of acid hydrolysis of *T. muelleri*

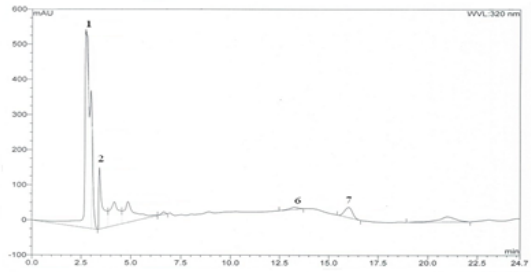


Fig. (6): HPLC chromatogram of alkaline hydrolysis of *T. muelleri*

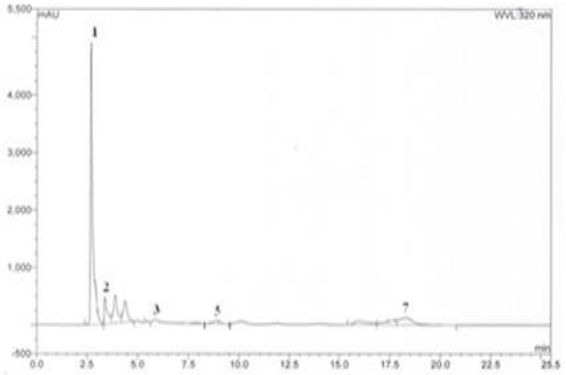


Fig. (7): HPLC chromatogram of alcoholic extract of *T.* myriocarpa

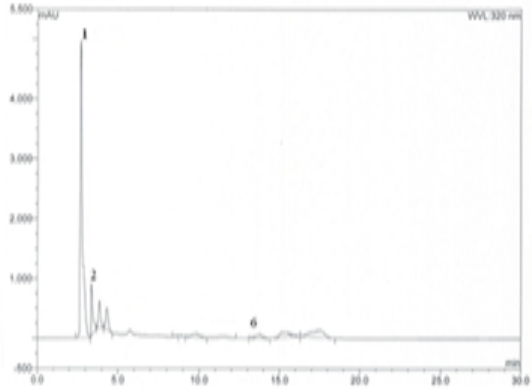


Fig. (8): HPLC chromatogram of acid hydrolysis of *T. myriocarpa*

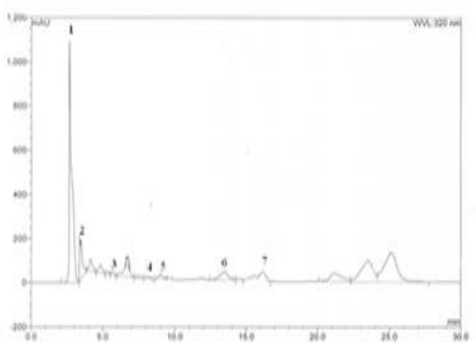


Fig. (9): HPLC chromatogram of alkaline hydrolysis of *T. myriocarpa*.

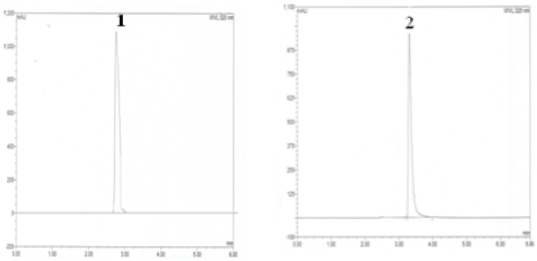


Fig. (10): HPLC chromatogram of cinnamic acid standard
Fig. (11): HPLC chromatogram of gallic acid Standard

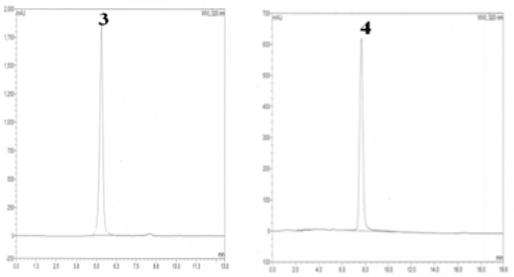


Fig. (12): HPLC chromatogram of chlorogenic acid standard
Fig. (13): HPLC chromatogram of caffeic acid standard

4.4. Results of determination of flavonoids:

4.4.1 Results of determination of total flavonoids by UV spectroscopy:

The total flavonoids of the 70% methanolic extracts of both *T. muelleri* and *T. myriocapa* leaves were determined colorimetrically by using the aluminum chloride method and quercetin as the reference material.

The total flavonoid content of extracts could be calculated from the following equation: Y = 0.021X – 0.011 as shown **in Fig. (17)** where Y=absorbance, X= corresponding concentration µg/ml. The flavonoid content of the leaves of *T. muelleri* and *T. myriocapa* was 17.9µg quercetin/mg crude extract, and 8.195 µg quercetin/mg crude extract respectively, calculated as quercetin equivalent. So flavonoid content of *T. muelleri* increases 2.18 fold from that of *T. myriocarpa.*

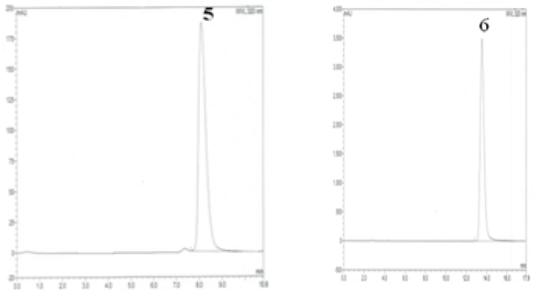


Fig. (14): HPLC chromatogram of synergic acid standard
Fig. (15): HPLC chromatogram of P-coumaric acid standard

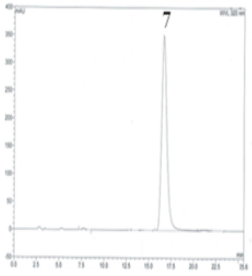


Fig. (16): HPLC chromatogram of ferulic acid standard

4.4.2. Results of a qualitative and quantitative investigation of flavonoids by LC mass:

Flavonoids in the ethyl acetate fractions of both plants were analysed as shown in figs. (21-22) and total ion chromatograms of HPLC-ESI-MSn (n = 2) systems were used to confirm peak Identities. The abundances of ions originating from flavonoid molecules were chosen based on preliminary studies or literature data; a complete structural characterization of these compounds was not only based on mass spectra, but also the combination of MS, MS/MS, and the comparison of Rt and MS data with similar reported data. The LOQ values were calculated as 22.95 ng/ml of rutin and 112.57 ng/ml of quercetin. Also, the LOD values were calculated as 6.89ng/ml of rutin and 33.77 ng/ml of quercetin.

Table (1): Results of HPLC analysis of phenolic acids (free and combined) of two *Terminalia* species

Phenolic acid Standards	The retention time of phenolic acid standards	Compound concentrations (µg/mg of crude extract)					
		T. muelleri			*T. myriocarpa*		
		fraction A	fraction B	fraction C	fraction A	fraction B	fraction C
Cinnamic acid	2.75min.	22.6	6.6	1..3	61	27	1.8
Gallic acid	3.41min.	6.4	3.4	0.53	17.3	7.7	0.46
Chlorogenic acid	5.31 min.	0.23	_	_	0.17	_	0. 0184
Caffeic acid	7.67 min.	_	0.0188	_	_	_	0. 0038
Synergic acid	8.1 min.	_	0.154	_	10.4	_	0.36
P. Coumaric acid	13.4 min.	_	0. 049	0. 0036	_	0. 078	0.035
Ferulic acid	16.67 min.	0. 035	_	1.74	0.123	_	0. 021
Unknown		8 peaks	3 peaks	5 peaks	5 peaks	7 peaks	7peaks
Total		29.3	10.2	3.6	89	34.8	2.7
% of phenolic acids		19.5%	6.8%	2.4%	76.7%	29.98%	2.3%

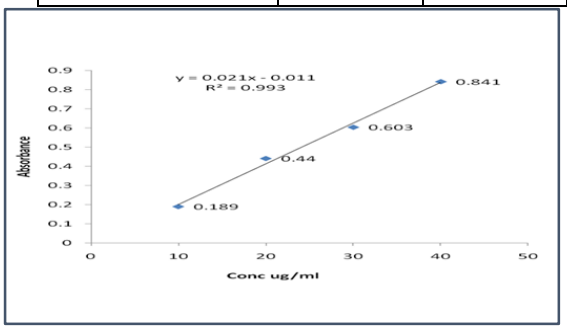


Fig. (17): Calibration curve of quercetin

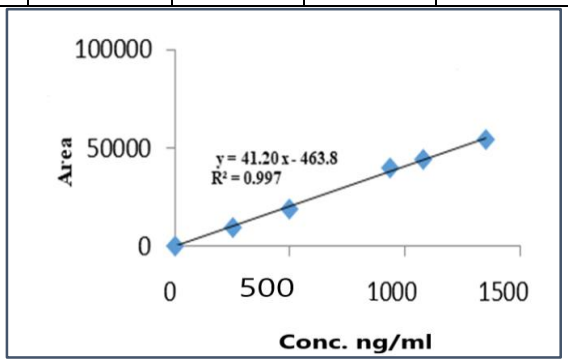


Fig. (20): Calibration curve of quercetin

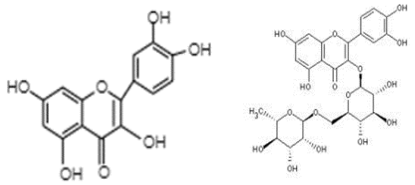


Fig. (18): Structure of quercetin and rutin

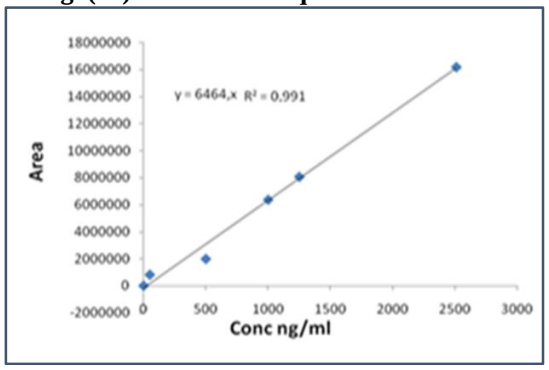


Fig. (19): Calibration curve of rutin

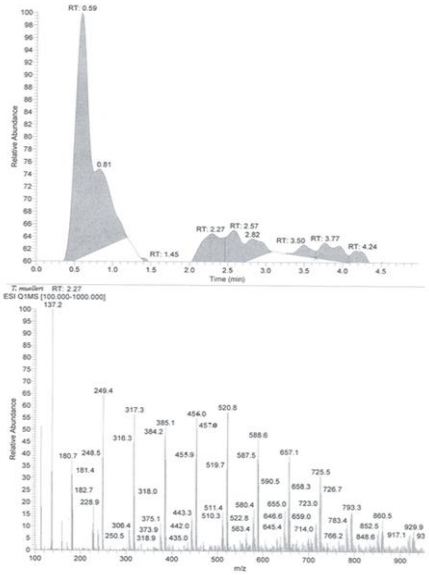


Fig. (21): LC/MS chromatogram of *ethyl acetate* fraction of *T. muelleri*

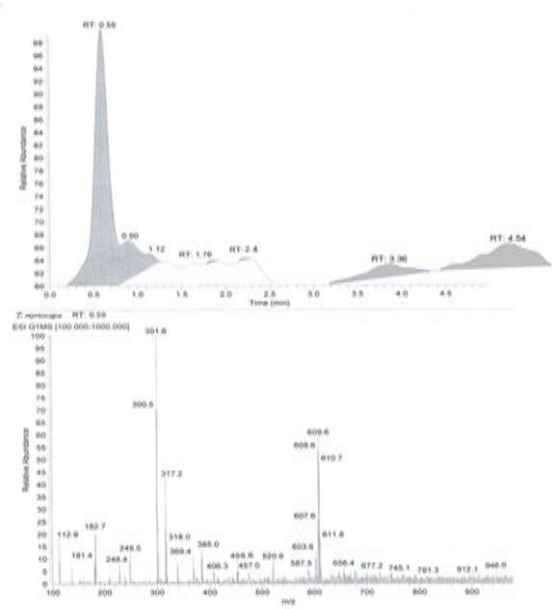
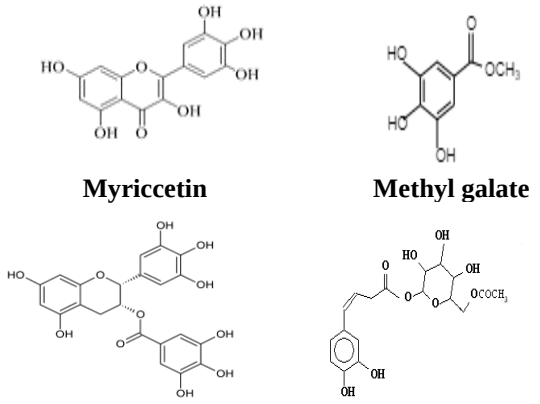


Fig. (22): LC/MS chromatogram of *ethyl acetate* fraction of *T. myriocarpa*



Myriccetin **Methyl galate**

Epigallocatechin gallate **Caffoyl acetyl hexoside**

Fig. (23): Structure of identified flavonoids

Table (2): Quantitative estimation of flavonoids in *T. muelleri* and *T. myriocarpa*

Authentic standards	*T. muelleri*			*T. myriocarpa*		
	Average amount (µg/mg of ethyl acetate	Average amount (µg/g of crude extract)	Recovery %	Average amount (µg/mg of ethyl acetate	Average amount (µg/g of crude extract)	Recovery %
Rutin	1.18	0.40	98.85 %	81.83	27.28	98.85 %
Quercetin	20.53	6.84	98.39 %	108.6	36.20	98.39 %

Fragmentation pattern of the identified compounds:

I- MS^2 of gallic acid: Compound showed MS at [M-H] at m/z 183 with major fragment 124 and 168 compounds which represent a loss of methyl fragment [M-15], it was identified as methyl gallate **(Pfundstein *et al.*, 2010).** The structure is illustrated in **Fig. (23)**.

II- MS^2 of O- glycosides: Compound showed MS at m/z 385 $[M-H]^-$ which had 42 m/z larger than that of caffoyl hexoside of m/z 342 $[M-H]^-$ suggesting its acetyl derivative, attachment of the acetyl group to the hexosyl moiety was proved by the loss of 204 m/z (162 + 42) from the 100% peak to give the caffeic acid fragment 181$[A-H]^-$; a similar fragmentation was previously described by **(Moco *et al.*, 2006).** It was proposed to be a caffoyl acetyl hexoside structure, since standards of these compounds were not available, we depended on the literature data of a compound with a near structure and defined as caffoyl hexoside. The structure is illustrated in **Fig. (23**).

III- MS^2 of Flavan 3-ol: The compound showed MS at [M-H] at m/z 457 with major fragment 331.Compound represents a loss of gallate fragment [M-125], it was identified as epigallocatechin gallate (**Rio *et al.*, 2004**). The structure is illustrated in **Fig. (23)**. **IV- MS^2 of flavone:** Compound showed MS at m/z 318 $[M-H]^-$ which had 18 m/z larger than that of quercetin suggesting its hydroxyl derivative 299 (MS^2 $[M-18]^-$due to the loss of hydroxyl group. It was proposed to be myricetin. Since standards of these compounds were not available, we depended on the literature data of the fragmentation of a compound and defined as myricetin **(Sojka *et al.*, 2009).** The structure is illustrated in **Fig. (23).**

Table (3): Show the retention times (Rt) and masses of the identified compounds in both plant species

Identified compounds	Retention time	$[M-H]^-$	MS^2
Methyl gallate	2.27 min.	183	124 (100%), 168
Caffoyl acetyl hexoside	2.27 min.	385	181(100%)
Epigallocatechingallate	0.59 min.	457	331(100%), 289, 169
Myricetin	0.59 min.	318	272, 181, 152(100%)

HPLC-UV-MS/MS-ESI in the negative mode was used to assess flavonoid content in ethyl acetate extracts of *T. muelleri* and *T. myriocarpa*. The flavonoid compounds, rutin, quercetin, methyl gallate, and caffoyl acetyl hexoside were identified in the ethyl acetate fraction of *T. muelleri* leaf. While *T. myriocarpa* ethyl acetate fraction contains rutin, quercetin, epigallocatechin gallate, and myricetin. The quantification of the identified compounds, rutin and quercetin in ethyl acetate fraction of both plants by LC-MS/MS revealed that quercetin is the major (6.84, 36.20 µg /g crude extract) followed by rutin (0.40, 27.28 µg/g crude extract) in *T. muelleri* and *T. myriocarpa* leaves, respectively.

5. Discussion:

The results of the DPPH, FRAB, and total phenolic content are considerably increased with the increase concentration of the plant extracts, which is consistent with previous findings (**Singh *et al.*, 2011 and Gwatidzo *et al.*, 2018**). The antioxidant activity of phenolic substances is principally due to their redox properties, which can show a vital role in absorbing and neutralizing free radicals, quenching singlet, and triplet oxygen, or decomposing peroxides. For the measurements of the reductive ability, it has been found that the Fe+3-Fe+2 transformation happened in the existence of extract samples which was postulated previously by (**El-hela *et al.*, 1999**) that have observed a direct correlation between antioxidant activities and the reducing power of the examined plant extract. Furthermore, HPLC analysis of the qualitative and quantitative distribution for free and chemically bonded forms of phenolic acids in plant materials showed that phenolic acids are predominantly free form in *T. muelleri* and *T. myriocarpa* plants. The results of the present study revealed that the cinnamic acid and gallic acid are present in all analytical fractions of the two examined extracts, these findings are the same line with that of **Knaggs, 2001**. Phenolic compounds are considered to be the major phytochemicals involved in the antioxidant activity of plant materials. It has been reported that there is the correlation between antioxidant activity and phenolic content in plant extracts (**Claudia *et al.*, 2008; Javanmardi *et al.*, 2003 and Guleria *et al.*, 2010**).

Ethyl acetate extract from *T. muelleri* and *T. myriocarpa* were performed by HPLC MS/MSESI in negative mode to analyse flavonoid content. The results of the current study revealed that the flavonoid compounds (rutin, quercetin, methyl gallate, caffeylacetylhexoside) contained in the ethyl acetate fraction of *T. muelleri* leaf. While rutin, quercetin, epigallocatechin gallate, and myricetin were identified in the ethyl acetate fraction of *T. myriocarpa* leaf.

The quantification of the identified compounds, rutin and quercetin in ethyl acetate fraction of both plants by LC-MS/MS revealed that quercetin is the major (6.84, 36.2 µg/g crude extract) followed by rutin (0.45, 27.28 µg/g crude extract) of *T. muelleri* and *T. myriocarpa*, respectively.

Results of the present study revealed the presence of great amounts of polyphenolic compounds in the two *Terminalia* species that are contributed to the antioxidant activity of the two examined extracts. The antioxidant activity of the two extracts is based on the presence of many active ingredients such as flavonoids and phenolic compounds that agreed with **Javanmardi *et al.*, 2003; Claudia *et al.*, 2008; Sojka *et al.*, 2009; Guleria *et al.*, 2010 and Gwatidzo *et al.*, 2018.**

6. Conclusions:

Both species of *Terminalia* (*T. muelleri* and *T myriocarpa)* possess a valuable amount of polyphenolic compounds which renders these species exerted more antioxidant activity and can be used in traditional medicine and the pharmaceutical industry.

7. Conflicts of interest:

All authors declare that there is no conflict of interest.

8. Acknowledgments:

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