Study of Phytochemical, Antioxidant and Antimicrobial of Tribulus macropterus Boiss. var. arabicus (Hosni) Al-Hemaid & J. Thomas, Yemen

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Received: 31/01/2024
Revised: 18/02/2024
Accepted: 16/11/2024

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https://doi.org/10.20428/jst.v29i2.2264

Study of Phytochemical, Antioxidant and Antimicrobial of Tribulus macropterus Boiss. var. arabicus (Hosni) Al-Hemaid & J. Thomas, Yemen

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Abstract— The screening of the of the vegetative and fruit of 70% ethanol, 70% methanol and aqueous extracts showed the presence of tannins, carbohydrates, saponins, glycosides, flavonoids, terpenoids, coumarins and alkaloids. The vegetative part was found to possess comparatively higher values of alkaloids, glycosides, saponins, total phenolic and total flavonoids content. Of all the ethanol extracts tested from vegetative and fruit part exhibited strong antioxidant potential in scavenging DPPH, hydrogen peroxide, phosphomolybdenum assay and reducing power (IC50 values; 288.47 and 282.05 µg/mL, 58.64 mg ascorbic acid equivalents/g sample and 0.40; 400.1 and 381.07 g/mL, 38.63 mg ascorbic acid equivalents/g and 0.37, respectively). In ferric reducing assay the absorbance was found to increase with increase in concentration

The ethanol and aqueous extracts from vegetative and fruit parts of investigated plant parts had antibacterial activity in which vegetative showed highest values but did not show any antifungal activity.

Keywords— : Antimicrobial, Antioxidant, Phytochemical, Tribulus, Yemen

I. Introduction

The pharmacological usefulness of plants has become larger and very important because the variety of plants are a treasure house of potential drugs and significant sources for new pharmaceuticals and medicinal activities [1]. Products from plants have been part of phytomedicines possess interesting biological activities and can be obtained from barks, leaves, flowers, roots, fruits, seeds [2]. *Tribulus macropterus Boiss.* var. *arabicus* is prostrate or decumbent, woody based subshrub, 50-100 cm tall, which is endemic plant in Arabian peninsula: Saudi Arabia, United Arab Emirates, Qatar, Oman, and Yemen [3, 4, 5].

Yemen's flora is very rich and characterized by a strong endemism due to the geographical structure and various climates [6]. *T. macropterus var. arabicus* which belong to the family *Zygophyllaceae* is commonly known as Zhar or Qayoob [3]. *Tribulus* species have been recorded to have numerous uses as traditional medicine for urinary infections, Kidney stones, increased muscle strength, sexual potency, heart diseases and cough. It is considered invigorating stimulant, aphrodisiac, and nutritive. A minority of traditionally used medicinal plants have been evaluated for their pharmacological and chemical properties.

Up to date, the plant was not been investigated toward documenting its ethnomedicinal uses and establishing its chemical constituents, so the aim of this work was to study the phytochemical composition, antioxidant and antibacterial activity of vegetative parts and fruits of *T. macropterus var. arabicus.*

II. Materials And Methods

1. Collection and identification of plant material

The plant parts (vegetative part and fruits) of T. macropterus var. arabicus (Family: Zygophyllaceae) were collected from Toor Al-Baha, Lahej province, south Republic of Yemen at flowering stage. The plant was authenticated by a taxonomist, Prof. Dr. Othman Saad Saeed Al-Hawshabi, Biology Department, Faculty of Education, University of Aden, Yemen.

Figure1: Images of parts of plant T. macropterus var. arabicus (zygophyllaceae)

2. Chemicals

DPPH (2, 2-diphenyl-1-picrylhydrazyl), ammonium molybdate, Folin-Ciocalteu's reagent, dimethyl sulfoxide (DMSO), hydrogen peroxide (H_2O_2), quercetin, gallic acid,

ascorbic acid, aluminum chloride, potassium acetate, ciprofloxacin, and cefotaxime were purchased from Sigma-Aldrich. The solvent and all reagents used in the analysis were of analytical grade.

3. Micro-organisms

Four bacterial strains were isolated clinically according to method of [7] from vaginal discharge were (*Escherichia coli*, *Enterococus* spp., *Staphylococcus auras* and Bacterial vaginosis), one fungi (*Candida albicans*) was also isolated clinically diagnostic by a microbiologist, Professor Khaled Nasher Qhatan Salem in the Quality Control Lab – Microbiology Dept. - Supreme Board of Drugs and Medical Appliances.

4. Plant material

Vegetative parts and fruits were carefully washed with tap water and rinsed with distilled water. All plant materials were cut into small parts and then air-dried under shade at room temperature for five weeks then were crushed and ground into coarse powder with mortar and pestle then stored in airtight bottles for further analysis.

5. Extraction of plant

Aqueous extraction: 70 g of coarsely powdered sample was macerated with double distilled water taken in a conical flask. Magnetic stirrer was used to mix the solution at room temperature for 24 hours. After shaking, they were filtered with muslin clothes, centrifuged at 2500 for 15 min and again filtered with filter paper (Whatman No.1). The filtrate was then concentrated by a rotary vacuum evaporator (under reduced pressure at 60 °C). The dried crude extracts were stored at 4 °C until use [8].

Alcohol Extraction: The dried powdered vegetative part and fruit were subjected to individually Soxhlet extraction by various solvents namely ethanol and methanol 70% were used. A 70g-sample was packed in soxhlet apparatus subjected to continuous hot percolation for 24 h using 500ml of a solvent (ethanol and methanol). Then, the solvent was concentrated under vacuum using a rotary evaporator and then dried on a water bath to complete dryness [9].

6. Phytochemical Analysis

An aliquot of each extract (1mg/mL) obtained from vegetative part and fruit of *T. macropterus* var. *arabicus* was subjected to qualitative phytochemical analysis to ascertain the presence of secondary metabolites such as : alkaloids, coumarins, phenols, flavonoids [10]; tannins, triterpenoids and steroids [11]; carbohydrates, glycoside and amino acid & protein [12] and saponins [13] respectively.

7. Determination of Alkaloids

Alkaloids content of the plant sample was determined using the method described [14]**.** 20 g of the plant samples were extracted for 24 h with 100 ml of diethyl ether. The residue (on filter paper) was re-extracted again with 100 ml of 80 % ethanol for 24 h. The resulting extract was dissolved in 5%HCl, centrifuged (15min) and the aqueous portion was transferred to a new tube and basified with NH_4OH (pH 8-10). The aqueous (basic) portion was extracted with $CHCl_3$ three times and then concentrated under reduced pressure. Each sample was dried and weighed to determine the amount of alkaloid residues and expressed as a percentage of weight of sample analyzed.

8. Determination of Terpenoids

Dried plant powder 100 mg was taken and soaked in 250 mL of ethanol for 24 hour .The extract after filtration, was extracted with 10mL of petroleum ether using separating funnel. The ether extract was separated in pre-weighed glass vials and waited for its complete drying. Ether was evaporated and the yield of total terpenoids contents was calculated as percentages [15].

9. Determination of Saponins

Five grams of plant sample was dispersed in 50 ml of 20% v/v ethanol prepared in distilled water. The suspension was heated over hot water bath for 4 h with continuous stirring at 55°C. The mixture was filtered and the residue re-extracted with another 50 ml of 20% ethanol. The combined extracts were reduced to 20 ml over hot water bath at about 9°C. The concentrated solution obtained was shaken vigorously with 10 ml of diethyl ether in a 250ml separating funnel; the aqueous layer was collected while the ether layer was discarded. The purification process and repeated. Twenty milliliter of but-1-ol was added to the filtrate and then washed twice with 10 ml of 5% w/v aqueous sodium chloride. The whole mixture was heated to evaporation on hot water bath and later oven dried at 40°C to a constant weight. The saponin content was calculated as percentage [16].

10. Determination of Glycoside:

Glycoside content of the plant sample was determined using the method [17].

5 grams of the plant powder was added to 80 ml of 20% ethanol, then was placed on the shaking device for 6 hours. It is then filtered. The extraction process of the residual deposit after filtration was repeated in the same way for two more times. Filtrate extract was collected from the three processes and shook vigorously with 25 ml of chloroform in a separating funnel, The chloroform extraction process was repeated for five times again. The chloroform extract was collected from the six processes and then evaporated in the rotary evaporator until drying. Solvent deposit

https://doi.org/10.20428/jst.v29i2.2264

residual in a mixture of chloroform and methanol (1: 1), the solution was then filtered and then dried to dry on a hot water bath until the weight was stable. The resulting precipitation represents the total glycosides in the studied plant sample and calculated as percentage.

11. Determination of Total Phenolic Content

The total phenol content was determined as reported by [18]. A 1ml of each extract (1 mg/g) was mixed with 9 mL of distilled water in a 25 mL volumetric flask. 2.5 mL of a 10 fold dilute Folin-Ciocalteau phenol reagent (1:10 with water) was added. After 5 min, 10 mL of 7.5% Na_2CO_3 solution was added to the mixture and made up to the mark with distilled water. The mixture was incubated in the dark for 90 minutes at room temperature. A set of standard solutions of Gallic acid (25-175 µg/mL) were prepared in the same manner as described for the extracts. The absorbance was taken at 700 nm using a SINCO PDA UV-VIS spectrophotometer. All the samples were analyzed in three replications. The total phenol was determined with the help of standard curve prepared from pure phenolic standard (Gallic acid) and expressed as mg Gallic acid equivalents (GAE) /g dray weight of plant extract. Determination performed in triplicates (n=3).

12. Determination of Total Flavonoid Content (TFC)

The TFC of the different plant extracts was determined by Aluminum Chloride Colorimetric Method as described in [19]. A 0.5ml of 1mg/ml of each extract/standard stock solution was mixed with 0.1ml of 10% aluminum chloride and 0.1ml of potassium acetate (1M). In this mixture, 4.3ml of 80% solvent was added to make 5ml volume. This mixture was vortexed and the absorbance was measured spectrophotometrically at 430 nm. The sample blank was prepared similarly by replacing aluminum chloride with distilled water. The quercetin was used to make the calibration curve by preparation stock solution of 1mg/ml quercetin in solvent then diluted to different concentration (1.56, 3.125, 6.25, 12.5, 25, 50, 75, 100 µg/ml). the absorbance was measured at 430 nm. All measurements were done in triplicate. The results were expressed as mg of quercetin equivalent (QE)/g dry weight of plant extract.

13. Antioxidant Studies

DPPH Free Radical Scavenging Activity

The antioxidant activity of samples extracts (70%ethanol, 70% methanol and aqueous) was carried out according to the method described by [20]. Briefly, a 0.1mM solution of DPPH was prepared in Methanol, and 1ml of this solution was added to 3ml of the solution of all extracts in 80% methanol at different concentrations (1000, 500, 250, 125, 62.5 and 31.25 µg/ml). The mixture was shaken vigorously for 1 minute and allowed to stand at room temperature for 30 minutes. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm against blank sample with a spectrophotometer UV-Vis (Perkin Elmer-lambda 365) and compared to an ascorbic acid calibration curve. The mixture of DPPH and an ascorbic acid (100-3.125 µg/ml) was used as a positive control and the a test tube with 3ml of methanol and 1 mL of 0.1 mM DPPH solution serves as negative control. Extracts without DPPH were also used as blank solutions.

The percentage of inhibition was calculated using the following equation:

$$\textbf{\% inhibition} = \frac{\textbf{Abc} - (\textbf{Ab1} - \textbf{Ab0})}{\textbf{Abc}} \times \textbf{100}$$

Abc = absorbance of negative control, Ab1 = absorbance of extract with DPPH and Ab0 = absorbance of extract without DPPH (sample blank).

The amount of sample necessary to decrease the absorbance of DPPH by 50% (IC50) was calculated graphically for the different extracts' solutions of the plant parts in six different concentrations.

Hydrogen Peroxide Scavenging Activity (H2O2)

The principle of this method is there a decrease in absorbance of H_2O_2 upon oxidation of H_2O_2. The ability of extracts to scavenge hydrogen peroxide can be estimated according to the method described earlier by [21].

A solution of 40 mM H_2O_2 was prepared in 0.2 M phosphate buffer (pH 7.4). Aliquots (0.1 mL) of different extracts (1000, 500, 250, 125 62.5 and 31.25 µg/ml) in distilled water were transferred into the test tubes then 0.6 mL hydrogen peroxide solution was added to plant extracts and their volumes were made up to 4 mL with phosphate buffer. The identical reaction mixture without the sample serve as negative control, tubes were vortexed and, after 10 minutes, absorbance of the hydrogen peroxide was determined at 230 nm against a blank with a spectrophotometer UV-Vis (Perkin Elmer-lambda 365) and compared to an ascorbic acid calibration curve. Ascorbic acid (12.5, 6.25, 3.125, 1.56, 0.78 and 0.39 µg/ml) was used as a positive control and the sodium phosphate buffer solution without H_2O_2 was used as a blank.

The percentage of H_2O_2 scavenging by all extracts and standard compounds was calculated using the following equation:

$$\textbf{\% inhibition} = \frac{(\textbf{Ac}-\textbf{As})}{\textbf{Ac}} \times \textbf{100}$$

where Ac is the absorbance of the control and As is the absorbance in the presence of extract or other scavengers (ascorbic acid), each experiment was carried out in triplicates.

The IC50 was calculated graphically for the different extracts solutions of the plant materials in six different concentrations.

Ferric cyanide (Fe^{+3}) Reducing Antioxidant Power Assay

The ferric reducing antioxidant power (FRAP) method of [22] was used to measure the reducing capacity of plant extracts. The reaction mixture contained 1.0 ml of various concentrations of extracts (100, 200,300, 400, 500 and 600 µg/mL), 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The mixture was incubated at 50°C for 30 min and the reaction was terminated by the addition of 2.5 ml of 10% trichloroacetic acid, followed by centrifugation at 3000 rpm for 10 minutes. 2.5 ml of the upper layer was mixed with 2.5 ml of deionized water and 0.5 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against blank with a spectrophotometer UV-Vis (Perkin Elmer-lambda 365. The reducing power ability of the sample is determined by Increased absorbance of the reaction mixture indicates grater reduction capability [23]. Ascorbic acid (0.39, 0.78,1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200 µg/ml) was used as standard for comparison.

14. Phosphomolybdenum assay

The total antioxidant capacity of the all extracts were determined by the phosphomolybdenum method [24]. A 0.3 ml extract was combined with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solution were incubated at 90°C for 90 minutes. Then, after cooling the absorbance of the solution was estimated at 695 nm using a spectrophotometer against the blank which has been accomplished higher Pharmaceutical Authority for quality control using Perkin Elmer UV/Vis lambda 365 Spectrophotometer. Methanol (0.3 mL) in the place of the extract was used as the blank. The total antioxidant activity is expressed as the number of gram equivalent of ascorbic acid. The calibration curve was prepared by mixing ascorbic acid (0.39, 0.78,1.56, 3.125, 6.25, 12.5, 25, 50, 100 and 200ug/ml) with 3 ml of reagent solution.

15. Antimicrobial screening

Based on the results of phytochemical analysis, the antimicrobial screening of the plant parts was done using agar well diffusion method for aqueous and ethanol extracts.

The agar plates were swabbed (using sterile cotton swabs) with 24 hours old prepared inoculum of respective bacteria (*Escherichia col*i, *Enterococus* spp., *Staphylococcus auras* and Bacterial vaginosis then inoculum was allowed to dry for 10 min, the agar was prepared by dissolving 38 g of Muller Hinton agar in one-litter solution. 5 wells (6 mm diameter holes 25mm apart from one another) were made in each of the Muller Hinton agar plates by using sterile cork borer [25]. The different amounts of the extracts (240, 280, 320, 360, 400 mg/ml) for *T. macropterus* var. *arabicus*; were added to each of the 5wells. Reference standards Ciprofloxacin (0.1 mg/ml), Cefotaxim (0.6 mg/ml) and Clindamycin (0.04 mg/ml) were placed on the agar surface, served as positive controls. 5µl of the solvent dimethyl sulfoxide / deionized water added to wells served as negative controls. Petri plates were then allowed to stand at room temperature for 1 h and incubated at 37 °C overnight. Controls were run in parallel whereby solvent was used to fill the well. The plates were observed for zones of inhibition after 24 h and the results compared with those of the positive control. An average zone of inhibition was calculated for three replicates.

A modified Muller Hinton agar was recommended for the diffusion of antifungal agents through an agar gel as described in Clinical and laboratory standards institutes CLSI Approved standard. Mueller Hinton agar was supplemented with glucose to a final concentration of 2%. The addition of methylene blue to a final concentration of 5 µg/ml was done to enhance zone edge definition [26]. Then the same steps were done as the antibacterial assay. Use Nystatin (antifungal drug) was used as a positive control.

16. Statistical analysis

The data generated from quantitative assays for phytochemicals and antioxidant activity were statistically analyzed using a completely Randomized Design (CRD), with three replications. Means of treatments were compared using least Significant Differences (LSD) at 5% level, Using a program GenStat5 release 3.2. Results were expressed as the mean ± standard error of mean (SEM) for antimicrobial activity.

IV. RESULTS AND DISCUSSION

1. Qualitative analysis

Carried out on plant parts showed the presence of phytochemical constituents and the results are summarized in Table 1.

https://doi.org/10.20428/jst.v29i2.2264

Table 1. Screening of some phytochemicals in two parts of *Tribulus macropterus* var. *arabicus*

No	Phytochemical screening		*Tribulus macropterus* var. *arabicus* (Zygophyllaceae)					
			Vegetative part			fruit		
			70% EE	70% ME	AE	70% EE	70% ME	AE
1	Alkaloids	Wagner's test	+++	+++	+++	+++	+++	+++
		Mayer's test	+++	+++	+++	+++	+++	+++
		Dragendroff's test	+++	+++	+++	+++	+++	+++
2	Polyphenols	Ferric chloride test	+++	+++	+	++	+++	+
3	Flavonoids	Shinoda test	++	+++	++	+++	+++	++
		NaOH Test	++	+++	++	+++	+++	++
		Lead acetate	++	+++	++	+++	+++	++
		Aluminium solution test	++	+++	++	+++	+++	++
4	Saponins	Foam test	+++	++	+++	-	++	++
5	Sterols/ Triterpenes	Liberman test	+++	+++	-	+++	+++	-
		Salkowski test	+++	+++	+	+++	+++	+
6	Carbohydrate	Molish's test	+++	+++	+++	+++	+++	+++
		Fehling' test	+++	+++	+++	+++	+++	+++
		Bendict's test	+++	+++	+++	+++	+++	+++
7	Coumarine	Sodium Hydroxide test	+++	+++	+	+++	+++	+++
8	Glycosides	Keller Killani test	+++	+++	+++	+++	+++	+++
9	Tannins		++	+++	+	++	+++	+
10	Amino acids	Ninhydrine test	+++	-	+	-	-	+++
11	Protiens		+++	-	+	-	-	+++
12	Anthraquinone glycoside	Brotrager test	-	-	-	-	-	-
		Modified brontrager test	-	-	-	-	-	-
13	Resins		++	++	++	+	+	+

+++ = most intense, ++ = moderately intense, + = least intense, - = absent, EE = ethanol extract, ME = methanol extract, AE = aqueous extract.

It showed that tannins, carbohydrates, coumarins, glycosides, flavonoids, terpenoids and alkaloids were present in tested plant parts extracts. Anthraquinone glycosides were found to be absent in parts of *T. macropterus var. arabicus.* The absence of saponins and amino acid & protein from the ethanol extract of fruit and methanol extract of two parts. The preliminary phytochemical screening tests are undoubtedly useful in the detection of the bioactive principles, which is responsible for the therapeutic effectiveness of most medicinal plants and affirming the potential of providing useful drugs for human and animal use, and lead to the drug discovery and development [26].

2. Quantitative analysis of phytochemicals

The results of quantitative analysis on six major groups of phytochemical constituents in the medicinal plant were summarized and shown in Table 2 and 3.

The quantitative analysis revealed that vegetative part exhibited higher alkaloids, glycosides, and saponins contents (1.43±0.15, 0.35±0.05 and 8.80±0.23 %, respectively), than fruit (1.27±0.12, 0.17±0.06 and 5.50±0.22 %) but the maximum terpenoids content was observed in the fruit of plant (1.67±0.35 %). A statistically significant difference ($P \leq 0.05$) was observed among two parts of *T. macropterus* var. *arabicus* of alkaloids, terpenoids, and saponins. All these phytoconstituents are groups of secondary metabolites known to exhibit interesting biological and pharmacological activities and serve as the starting point in the development of modern medicines as well as had physiological activities [27].

Table 2. Phytochemical composition (g/g dry weight) of two parts of *Tribulus macropterus* var. *arabicus*

Parts of plant	Alkaloids (%)	Glycosides (%)	Saponines (%)	Terpenoids (%)
Vegetative part	1.433	0.350	8.80	1.267
Fruit	1.267	0.173	5.50	1.667

3. Total phenolic, flavonoids content

Different concentrations of gallic acid and quercetin fwere used for preparing standard curves for the determination of total phenolics (TPC) in Figure 2 (y=0.0044x−0.0049, R^2 = 0.993) and flavonoids content (TFC) in Figure 3 (y=0.0132x+0.0064, R^2 = 0.9995) respectively.

Table 3. Contents of Phenolics and flavonoids in various solvent extracts of two parts *Tribulus macropterus* var. *arabicus*

Extract sample	Ethanol extract	Methanol extract	Aqueous extract
Total Phenolic Content (mg/g GAE) in vegetative part	53.69±1.62	37.17±1.15	32.78±1.25
Total Phenolic Content (mg/g GAE) in fruit	21.34±0.4	29.29±2.17	18.39±1.49
Total flavonoid Content (mg/g QE) in vegetative part	36.423±2.24	25.36±1.95	Nd
Total flavonoid Content (mg/g QE) in fruit	4.00±0.15	4.30±0.42	Nd

Values of TPC and TFC are obtained as mean ± SD. Nd = not detected

Table 3 showed the TPC and TFC in three different solvents used. The value obtained for the concentration of TPC revealed that vegetative part has higher amount of TPC compared to fruit part. The amount of total phenolic varied in the different extracts, ranging from 32.78±1.25 to 53.69±1.62 mg GAE/g dry extract in vegetative part. The highest amount of phenolic was in 70 % ethanol extract (53.69±1.62 mg GAE/g extract dry extract. While water was lowest in phenolic (32.78±1.25 mg GAE/g dry extract). In fruit, the highest amount of phenolic was in 70% methanol extract (29.29±1.17 mg GAE/g dry extract) followed by 70% ethanol, aqueous (21.34±0.4 and 18.39±1.5 mg GAE /g) respectively.

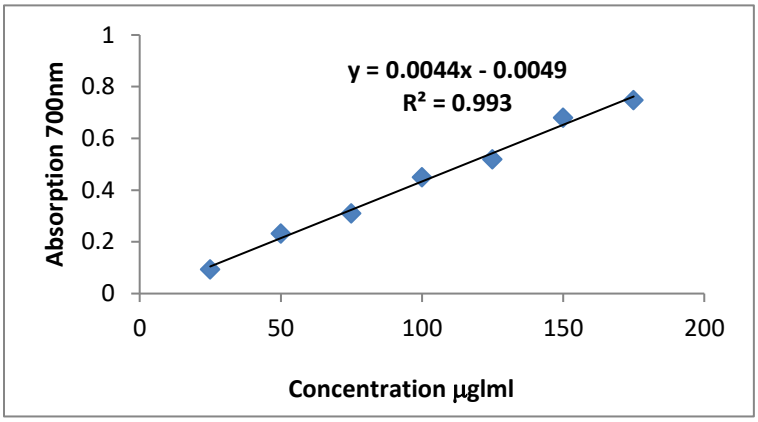

Figure 2: Construction of gallic acid standard curve

High solubility of phenols in polar solvents provides a high concentration of these compounds in the extracts [28], alcohol in various ratios used to minimize selectivity in that they extract all soluble constituents.

For the amount of flavonoids, a smallest quantity was in fruit and was not detected in aqueous extract of vegetative and fruit parts of plant. The concentration of flavonoid in plant extract is in agreement with results in case of TPC that was (36.42±2.24 and 25.36±1.95 mg/g) in ethanol and methanol extracts of vegetative part while (4.30±0.42 and 4.00±0.15 mg/g) in methanol and ethanol extracts of fruit; the aqueous extract did not contain flavonoids.

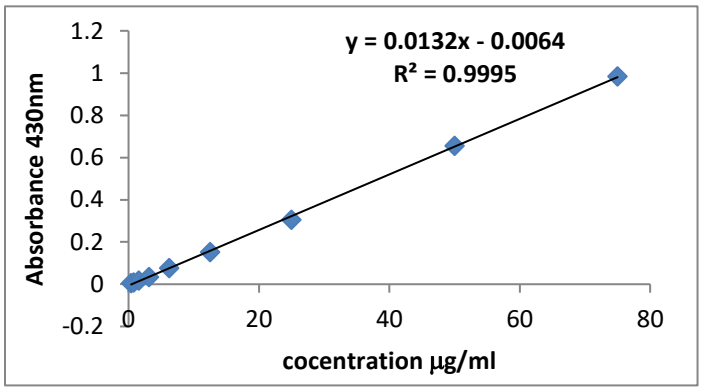

Figure 3: Construction of quercetin standard curve

Polyphenol compounds have attained because of their antiviral, antifungal, antibiotic, antitumor, anti- inflammatory, antimutagenic and antioxidant activities [29, 30]. The flavonoids and phenolic acids are known to possess antioxidant activities due to the presence of hydroxyl groups in their structures [31].

https://doi.org/10.20428/jst.v29i2.2264

4. In vitro antioxidant activity

Table 4 presents the antioxidant capacity of various extracts obtained from vegetative part and fruit of *T. macropterus* var. *arabicus*, examined using four different assays based on different chemical reaction mechanisms. The assessments of antioxidant properties of natural compounds are very important because of their uses in medicine, food and cosmetics [32].

DPPH is a stable nitrogen-centered free radical commonly used for testing radical scavenging activity of the compound or plant extracts. The antioxidant activity of 70% ethanol, 70% methanol and water of vegetative part and fruit showed to possessed scavenging properties but to varying degrees. A statistically significant difference ($p \leq 0.05$) was observed in the DPPH radical scavenging capacities of two parts of *T. macropterus* var. *arabicus*. The better activity was obtained from vegetative part with IC50 value (288.47±2.7, 314.1±1.5 and 383.1±2.1 µg/ml) i.e. an increased magnitude in the order of the ethanol > methanol > aqueous, respectively while fruit part exhibited little free radical scavenging capacity (400.1±2.5, 444.31±3.2, 987.85±3.4 µg/ml) at the same order of effectiveness, their scavenging ability was found to be lower than that of ascorbic acid (2.36 ± 0.5 µg/mL) Fig. 4. all parts of *T. macropterus* var. *arabicus* showed lower percentage of DPPH radical inhibition and higher IC50 values as compared to this ascorbic acid taken as standard antioxidants (2.36 ± 0.5 µg/mL).

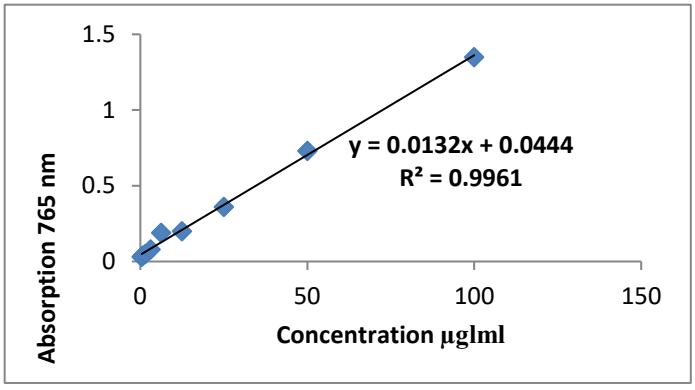

Figure 4: Construction of ascorbic acid standard curve

It is well known that DPPH scavengers have a wide range of biological activities such as lipid peroxidation inhibitory action, radioprotective activity and so on [33].

The IC50 values of the selected parts extracts for H_2O_2 scavenging activity are presented in Table 4. Vegetative part extracts showed stronger hydrogen peroxide radical scavenging activity (IC50 282.05±2.9, 290.14±2.5, 347.30±3.2 µg/ml, respectively, $p \leq 0.05$ versus ascorbic acid in the order ethanol > methanol > aqueous that the standards ascorbic acid gave IC50 value of (4.71±1.1 µg/ml). The scavenging activity for hydrogen peroxide Of fruit (IC50 381.07±4.0, 492.61±3.6, 683.68±3.4 µg/ml) in the order ethanol > methanol > aqueous. The potassium ferricyanide reduction assay measures the antioxidant effect of any substance in the reaction medium as reducing ability, antioxidant substance can reduce Fe^3 to Fe^2 through an electron donation process. Fe^2 in its complex form has Perl's Prussian blue color, which is monitored by recording mixture absorbance at 700 nm. High Fe^2 level indicated by increase in the absorbance of mixture demonstrates the increase in the reduction capacity (Benzie 1996). The reducing potential of tested parts of *T. macropterus* var. *arabicus* extracts increased as the extract concentration increases the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of antioxidant samples. The values of absorbance for tested parts showed vegetative part had higher reducing power compared to fruit part**.** The values of absorbance were determined to be range from (0.008±0.0 2 – 0.40±0.01), that revealed ethanol and methanol had stronger reducing power. Table 4 shows reducing potential of two parts of *T. macropterus* var. *arabicus*. Beyond the fact that FRAP assay is inadequate for the determination of hydrophilic (water soluble) antioxidants [34]. The statistical analysis of results showed a significant difference ($p \leq 0.05$) in the reducing abilities of two parts of plant.

Total antioxidant capacity of antioxidant compounds in parts extracts of *plant* was assessed through phosphomolybdate assay included the reduction of Mo (VI) to Mo (V) determined from regression equation of calibration curve (**$y = 0.0108x + 0.0316$, $R^2 = 0.9982$**) of Ascorbic acid (0.39–100 µg/mL) and expressed as equivalents of ascorbic acid (mg/g of extract) at 500 µg/ml sample Figure 4. The statistical analysis of the results showed a significant difference ($p \leq 0.05$) among two parts of *T. macropterus* var. *arabicus* regarding the more effective in reduction of Mo (VI) to Mo (V) extracts was shown by vegetative part as shown in Table 4 (58.64±2.2, 51.11±1.8, 24.09±1.5 mg ascorbic acid equivalents/g sample) while fruit (38.63±1.3, 32.27±1.1, 30.15±0.8 mg ascorbic acid equivalents/g sample), ethanol followed methanol showed Maximum antioxidant activity while aqueous extract produced the minimum yield. The results showed that all of the studied parts of *T. macropterus* var. *arabicus* are good source of phenolic compounds due to high TF and TPA content. Natural antioxidant mainly comes from plants in the form of phenolic compounds such as flavonoid, phenolic acids, tocopherols etc.[35, 36]. They are very effective antioxidant and show strong anticancer activities[37].

Table 4. Antioxidant powder of different extracts of *Tribulus macropterus* var. *arabicus* for various antioxidant systems

Part	Solvent	IC_{50} Of DPPH (µg/ml)	IC_{50} Of H2O2 (µg/ml)	Phospho-molybdenum (mg/ml)	Reducing power (600 ug/ml)
Vegetative part	Ethanol	288.47±2.7	282.05±2.9	58.64±2.2	0.40±0.01
	Methanol	314.10±1.5	290.14±2.5	51.11±1.8	0.39± 0.004
	Aqueous	383.10±2.1	347.30±3.2	24.09±1.5	0.30±0.006
Fruit	Ethanol	400.10±2.5	381.07±4.0	38.63±1.3	0.37±0.008
	Methanol	444.31±3.2	492.61±3.6	32.27±1.5	0.34±0.005
	Aqueous	987.85± 3.4	683.68±3.4	30.15±0.8	0.12± 0.004

It is well known that the antioxidant potential of the plant material usually appears to correlate with the phenolic content [38]. The antioxidant activity of plant parts extracts was mainly due to redox properties of phenolic compounds which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. [39].

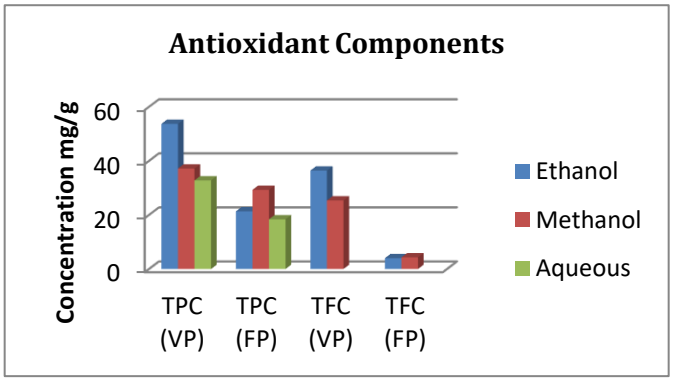

Figure 5. Contents of phenolics and flavonoids in various solvent extracts of *Tribulus macropterus* var. *arabicus* leaves.

The analyses in the present work of correlation between the phenolic content and antioxidant activity measured in the four assays varied with the material used (Table 5). The highest positive correlation for the extracts from fruit material has been noted (r = 0.981, 0.994, 0.782 and 0.999 for the relationship between the content of phenolics and the DPPH, H_2O_2, RP and Phosphomolybdenum assay respectively). the correlations between the antioxidant activities and the phenolic contents were also positive in vegetative material and antioxidant systems.

Table 5. Correlation among total phenolic compound content and antioxidant activities **(DPPH, H_2O_2, RP and Phospho-molybdenum assay)** of studied plants.

	Phenols/ DPPH	**Phenols /H_2O_2**	**Phenols /RP**	**Phenols/ Phosphomolybdenum**
***T. macropterus* var. *arabicus* fruit**	0.981	0.994	0.782	0.999
***T. macropterus* var. *arabicus* vegetative**	0.998	0.996	0.728	0.803

5. Antimicrobial activity

In this study, the antimicrobial activity of the ethanol and water extracts from two parts of *T. macropterus* var. *arabicus* were investigated against *Escherichia coli*, *Enterococus* spp., *Staphylococcus auras*, Bacterial vaginosis, and *Candida albicans*. Vegetative part showed best antibacterial activity than fruit, which certainly indicates that vegetative part of *T. macropterus* var. *arabicus* contain higher concentrations of active antibacterial agents as saponins, alkaloids, phenol and flavonoid. We have found that the ethanol and water extracts of two parts of *T. macropterus* var. *arabicus* did not not show any antifungal activity. However, the main components of *Tribulus* genus are saponins [40, 41] but the ineffectiveness of the saponins on fungus such as *Candida albicans*, may be as a result of the protective effect of the microbial coat [42].Vegetative part extracts exhibited significant antibacterial activity of which water extract had higher activity with increasing concentration than that ethanol extract except against *Staphylococcus auras* showed no activity.

The inhibitory zone for water extract of vegetative part scored as 14.73±0.25, 12.77±0.26 and 16.07±0.25 mm against *Escherichia coli*, *Enterococus* spp., and Bacterial vaginosis respectively at concentration 400 mg/ml. The zone of inhibition ethanolic extract was 6.5±0.5, 7.5±0.5, 8.83±0.29

https://doi.org/10.20428/jst.v29i2.2264

and 10.67±0.58 for the tested organism *Escherichia col*i, *Enterococus* spp., *Staphylococcus aura*sand Bacterial vaginosis respectively at the same concentration.

The antibacterial activity of fruit was week and it was not much effective against used human pathogens, it exhibited activity only against *Staphylococcus auras* and *Enterococus* spp. bacteria and nail activity against *Escherichia col*i, Bacterial vaginosis.

The inhibitory zone for ethanol extract was scored as 9.23 and 10.1 mm; 8.73 and 10.1 mm against *Staphylococcus auras* and *Enterococus* spp. at 360 and 400 mg/ml, respectively. Whereas water extract showed activity only against *Staphylococcus auras* at all concentrations (14.37 – 18.37 mm) while was not showed activity against *Enterococus* spp. Table 6.

The comparative antibacterial activity of ethanolic and aqueous extracts of vegetative and fruit of *T. macropterus* var. *arabicus* and standard antibiotics against tested microorganisms is shown in the Table6 and 7.

Result in this work for ethanol extract of *T. macropterus* var. *arabicus* has been confirmed with [43, 44, 45] on the antibacterial activity of methanol extract of the same genus Turkish and Iranian *T. terrestris* against selected bacteria and agreement with [44] for moderate activity of Iraqi *T. terrestris* against *Staphylococcus auras and Escherichia col*i.

Table 6. Antibacterial activity determined as zone of inhibition (mm) of selected plant extracts against selected bacterial strains

Species	**concentration (mg/ml)**	**inhibition zone**				
		Escherichia coli	***Enterococcus* spp.**	***Staphylococcus auras***	**Bacterial vaginosis**	***Candida albicans***
Vegetative ethanol extract	240	3.33±0.58	3.67±0.58	4.5±0.5	6.00±0.5	--
	280	4.67±0.29	5.33±0.29	6.17±0.29	6.5±0.4	--
	320	5.17±0.29	5.83±0.29	6.5±0.58	7.33±0.58	--
	360	6.00±0.5	7.00±0.5	7.67±0.5	9.17±0.76	--
	400	6.5± 0.5	7.5±0.5	8.83±0.29	10.67±0.7	--
Fruit ethanol extract	240	--	4.8±0.26	7.5±0.5	5.5± 0.5	--
	280	--	6.2±0.12	8.0±0.25	5.83±0.11	--
	320	--	6.77±0.25	8.73±0.15	6.5±0.25	--
	360	--	8.73±0.25	9.23±0.36	7.47±0.15	--
	400	--	10.1±0.36	10.1±0.21	8.73±0.36	--
Vegetative aqueous extract	240	10.73±0.25	9.5±0.5	--	12.5±0.25	-
	280	11.77±0.64	10.47±0.5	--	13.1±0.64	-
	320	12.1±0.36	10.77±0.26	--	13.77±0.36	-
	360	13.37±0.32	12.37±0.40	--	15.07± 0.32	-
	400	14.73±0.25	12.77±0.26	--	16.07± 0.25	--
Fruit ethanol extract	240	--	--	--	--	--
	280	--	--	--	--	--
	320	--	--	--	--	--
	360	--	--	--	--	--
	400	--	--	--	--	--

Table 7. Sensitivity test (inhibition effect) of microbial tested aganist some antibiotic

Antibiotic / **Microorganism**	**Ciprofloxacin**	**Cefotaxime**	**Clindamycine**	**Nystatine**
Escherichia coli	**19.67 ± 0.58**	**13.33 ± 0.58**	**11 ± 0.58**	**-**
***Enterococus* spp.**	**25.33 ± 0.58**	**27.33 ± 0.58**	**-**	**-**
Staphylococcus auras	**13.67 ± 0.29**	**20.67 ± 0.58**	**26.17 ± 0.29**	**-**
Bacterial vaginosis	**15.93 ± 0.12**	**11.3 ± 0.29**	**10.33 ± 0.58**	**-**
Candida albicans	**-**	**-**	**-**	**25.67 o.58**

VII. CONCLUSIONS

Data obtained in this study revealed the main phytochemical groups (sterols, terpenoids, cardiac glycosides, alkaloids, flavonoids and tannins) presented in the two parts of *T. macropterus* var. *arabicus* and illustrated their significant antioxidant and antibacterial activity. These data provide scientific justification for its usage in the treatment of various illnesses. Further work for phytochemical and pharmacological investigation is required to establish their efficacy and safety as well as to search further biological activities.

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