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Assessment of phytochemical screening, total phenolic content, antioxidant activity of leaves and stems extract from Adenocarpus bacquei and its essential oil antioxidant activity

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Abstract

The researchers' focus has recently been directed towards finding new natural antioxidants especially those originated from plants. This work is about the screening of phytochemical constituents and the variation of total phenolic and flavonoid content for different parts of Adenocarpus bacquei (leaves and stems) harvested from Morocco. It regards also extracting its essential oil , analyzing its chemical composition by gas chromatography-mass spectrometry (GC/MS) which reveals the presence of 25 compounds where the major one is the (+)-2-Bornanone (38.26%) followed by Eucalyptol (27.74%).Yet, this essential oil proved great antioxidant capacities measured with TAC and DPPH assays. On the other hand, the phytochemical analysis of Adenocarpus bacquei's leaves and stems showed the existence of principal groups of secondary metabolites (tannins, flavonoids, mucilage, oses and holosides sterols and triterpenes and combined antraquinons). Quantifying total phenolic and flavonoid content of different crudes extracts of leaves and stems of Adenocarpus bacquei was done using solvents with different polarity (hexane, dichloromethane, ethyl-acetate, ethanol and water). These crude extracts' total antioxidant activity and radical scavenging activity were respectively evaluated with the phosphomolybdenum and DPPH assays. Therefore, the results showed a simultaneous increasing in total phenolic content of crude extract and the solvent polarity. Yet, the aqueous leaves extract indicates the highest TPC. Also, the results show a significant positive correlation between TAC and DPPH assay in relation with the total phenolic and flavonoid content. These results revealed the powerful capacity of Adenocarpus bacquei's essential oil and crudes extracts which comes to conclude that Adenocarpus bacquei can be a natural resource of antioxidants.

Keywords: *Correlation, antioxidant activities, essential oil, physical-chemical characteristics.*

1. Introduction

For a long time plants were used for traditional medicine due to their therapeutic effects against various diseases, the plant-derivate products were also utilized considering how rich they are with antioxidant properties, their low toxicity and strong bioactivities [1]. Recently, scientific researches started looking for new natural antioxidants which are able to donate protons to postpone Free radical's increasing and postpone formation of reactive oxygen species in health. Those reactive species cause oxidative stress in systemic level and increase health problems such as cancer and cardiovascular diseases [2]. Some aromatic plants' crudes extracts are rich of total phenolic contents; they revealed a significant total antioxidant activity that could be used as a potential natural source of antioxidants to prevent oxidative stress [3]. The aim of this study was to glamorize both the phytochemical screening, to highlight the correlation between total phenolic contents and antioxidant activities that were achieved by TAC and DPPH assays of different crudes extracts from Adenocarpus bacquei's leaves and stems. Also this work studied the chemical composition of Adenocarpus bacquei's essential oil and its total antioxidant activities.

2. Materials and methods

2.1. Vegetal material

The various parts (leaves, stems) of Adenocarpus bacquei were harvested on April 2018 from South East Morocco. They were well dried under the shadow at room temperature for 15 days then reduced to fine powder separately for hydrodistillation and solvents extractions. The vegetal material that we are working on consists of Adenocarpus bacquei which belongs to Fabaceae's family, Magniliopsida's class and Adenocaprus's type.

2.2. Extraction of essential oil

The extraction of the essential oil was executed by hydrodistillation in a Clevenger device. Three distillations were executed by boiling for an hour and a half 100 g of fresh vegetal material with 1L of water in a 2L tank which is 60 cm high. This tank has a column coming out of it which is connected to a refrigerant. The essential oil that we got was then stocked in a dark place in a temperature of 4°C with the presence of anhydrous sodium sulphate. Afterwards it was diluted in methanol (1% v/v) before doing the CG and CG/SM analysis [4].

2.3. Chemical composition

GC-MS:

The identification of chemical components was carried out using mass spectrometer interfaced with a gas chromatograph in the common analysis center faculty of sciences Meknes, GC-MS: Agilent technologies 7890 B, equipped with Agilent 19091S-433 fused silica capillary HP-5MS column (5% phenyl Methylpolysiloxane, 30 m, 250 µm; film thickness 0.25 µm), coupled to mass spectrometer Agilent technologies 5977 A MSD (ion source βγ0°C, 70ev) GC oven initial temperature was 70 ◦C during 45 min under the following operation conditions: vector gas: Helium. The injector temperature was 250°C; split ratio of 80/1 was injected; helium was used as the carrier gas at 3L/min. Identification of components was done by comparison of the case registry number (Cas) and MS with the corresponding database (NIST library) and with mass spectral literature [5].

2.4. Determination of physicochemical indexes

Physical characteristics

Density

It is the relation between the mass of certain oil's volume at 20°C and that of distilled water with the same volume and temperature. It consists of weighing successively a volume equaling that of the distilled water and the essential oil studied at the temperature 20°C by using a pycnometer. It also consists of calculating the density by using the relation 1 below:

$D_{20}^{20} = (m_2-m_0)/(m_1-m_0)$ (1)

With : m_0: the mass of the empty pycnometer. , m_1: the mass of the pycnometer filled with distilled water., m_2: the mass of the pycnometer filled with the studied oil.

Chemical characteristics

Acid index

The acid indicator is the necessary amount of milligrams of Potassium Hydroxides (KOH) needed to neutralize the free acids contained in one gram of essential oil. Weighing 1 g of the studied oil in an Erlenmeyer .adding 30 ml of ethanol, the titrating is effectuated with the potassium hydroxides solution (0.1N) and some drops of phenolphthalein until we obtain a pink color [6]. The acid indicator's formula is:

$I_{ACID} = M \times V \times N/m$ (2)

With : M: molar mass, expressed in g/mole, of KOH (M=56.1g/mole). N: normality of the titrated solution KOH (0.1N). V: volume ml of titrated KOH. m: mass (g) of the taken essay .

Determination of the saponification index:

It is based on the necessary mass of potassium Hydroxid (KOH) needed to saponify esters and fatty acids, it is also needed to neutralize fatty acids that has not been esterifies that exists in 1g of essential oil [7].

$I_s = ((V_T - V_E) \times C_{HCl} \times 56.1)/ m$ (3)

- With : I_s: index of saponification. V_T: blank's volume in mL. V_E: test's volume in mL. C_{HCl}: Chloric acid concentration in mol/L. 56.1 (g/mol): molar mass of KOH. m: mass of analyzed essential oil in g.

Determination of Ester index

Ester index is the necessary mass of potassium Hydroxid KOH (mg) to saponify esters that has not esterifies in 1g of essential oil [6] It is calculated using the following formula:

Ester index = Saponification Index – Acid index (4)

2.5. Phytochemical screening

Qualitative tests based on coloration change or precipitation reactions admit to identify chemical family alkaloids, tannins, flavonoids, saponins, sterols tri-terpenes, anthrancenics and mucilage in each part of plant leaves and stems [8].

2.6. Extraction of crude extract

Preparation of extracts

A mass of 30 g of leaves or stems of Adenocarpus bacquei was extracted with different pure solvents (300 mL) hexane, dichloromethane, ethyl-acetate, and ethanol. Using the Soxhelt device until the solvent turned colorless again. Then, the mixtures were filtered and the solvents were evaporated by rotary evaporator and the yields were calculated [9].

Infusion

The aqueous extracts obtained by adding boiling distillated water to 30g to each plant's part during 6h. Then the mixture was filtered and the solvents were filtered and evaporated.

Extraction yield

$R\ \% = (M_1/M_0) \times 100$ (5)

With: M_1: mass of essential oil or crude extract (g). M_0: masse of vegetal material (g).

Instrument

All spectrophotometric data were treated using SHIMADZU UVmin-1240 UV-VIS spectrophotometer. Glass cuvette (1cm× 1cm× 4.5 cm).

Statistical analysis

The results are mentioned as mean ± standard deviation of three replicates. Statistical analyzes were performed using Microsoft Excel followed by one way-ANOVA. Pearson's table of correlation coefficients was used to determinate the interrelationships among TFC, TPC and antioxidant activities, Values were performed statistically significantly when $P<0.05$.

2.7. Determination of phenolic compounds contents

Total phenolic content

The total phenolic content was determined by the Folin-ciocalteu method [10]. Briefly 0.3mL at 1mg/mL of each crude extract was mixed with 1.5mL of Folin-ciocalteu's reagent (diluted by distilled water to 1:10 v/v) and 1.2 mL solution at 7.5 % of sodium carbonate (Na2CO3). The mixture was incubated at dark for 2 hours then the absorbance was read with spectrophotometer at 765nm. The TPC was calculated using regression equation from the calibration curve using gallic acid standard, and the data are results of three replicates ± standard deviation. The total phenolic content was expressed in mg of Gallic acid equivalents per gram of dry weight (mg GAE/g).

Total flavonoid content

The Total flavonoid content was determined by the technique of Olajire A and Azeez L [11]. 1ml (1mg/ml) solution of each extract was added to 2,4mL of distilled water and 0,3mL of 5% $NaNO_2$ (Sodium Nitrate), after 5 minutes we added 0,3mL of 10% AlCl3. The mixture allowed standing for 6 minutes incubate at dark, finally 1mL of NaOH (Sodium Hydroxide) (1M) was added and this mixture was up to 10ml with distilled water. After agitation, the Absorbance was read immediately at 510 nm with a spectrophotometer. The values are results of three replicates and the data are expressed in term of milligram equivalent of Quercitin per gram of crude extract (mg QE/g) ± standard deviation. These results were calculated from calibration of quercetin calibration.

2.8. Evaluation of antioxidant activity

The antioxidant activity has been studied by two different methods, the first one measures the totality of this activity using the phosphomolybdenum method, while the second one measures the extract's capacity of trapping radicals using the most stable radical which is the DPPH (2,2-diphenyl-1-picryl-hydrazyl). Ascorbic acid (AA) was used as the standard and results were expressed in milligram equivalent of AA per gram of crude extract (mg AA/g) ± standard deviation. The values are the average of three replicates.

Total antioxidant activity (TAC)

This procedure is a spectroscopic method to quantify the total antioxidant activity, which is based on reducing in an acid environment Mo (VI) that is a colorless solution to Mo (V) which is green. This activity was calculated using the method described by PRIETO et al. [12] NUR ALAM [13]. Briefly, 0,1ml of sample (1mg/ml) mixed with 1ml of reagents (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM of ammonium molybdenum).The tube was incubated at 95°C during 90min.after cooling, the solution's absorbance was measured at 695 nm [13]. The TAC's values are results of three replicates ± standard deviation. The TAC was expressed in mg of Ascorbic acid equivalents per gram of crude extract (mg AA/g).

The DPPH free radical scavenging activity

The hydrogen atom donation ability of different crudes extracts and essential oil of Adenocarpus bacquei was measured by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical scavenging assay [14]. In this test, the purple DPPH is reduced to a yellow component which is the 2,2-diphenyl-1-picryl-hydrazine. The color's intensity is inversely proportional to the reduced capacity of the present antioxidants [15]. The reaction was realized in a total volume of 2 ml containing 1950 µL of DPPH (0.024 g/L). The samples were incubated for 30 minutes and the absorbance was measured at 515 nm [16].

The Inhibition % is calculated as follows:

Inhibition % = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$ (6)

With : A_{blank}: the negative control Absorbance at 515nm. A_{sample}: the sample Absorbance at 515nm.

The extract's different concentrations which reduced the DPPH solution allowed the calculation of IC_{50}, a stronger antioxidant activity indicated by a low value of IC_{50}. The IC_{50} value corresponding of the sample's concentration is needed to trap 50% of DPPH in the solution test, the antioxidant capacity was determined from the IC50 value. The efficient concentration EC_{50} (mg/ mg of DPPH) was expressed of sample's concentration used for the test (mg/mL) in relation with the DPPH initial quantity (mg of DPPH/ mL of solvent).

$EC_{50} = IC_{50}$ /concentration of DPPH (7)

The Anti-Radical Power (ARP) value was determined as the efficient concentration's inverse value, that's why, the sample with a lower effective concentration EC_{50} (also lower IC_{50}) has the higher ARP value that's mean this sample has the greatest antioxidant activity.

$ARP = 100/ EC_{50}$ [17]. (8)

3. Results and discussion

3.1. Extraction of essential oil

Extraction of Adenocarpus bacquei's essential oil using the Clevenger device shows a rending equals to 1.4 ± 0.02 % after tree hydrodistillation.

3.2. Determination of physicochemical characteristics

The results of the essential oil's physicochemical characteristics obtained are summarized in (see **Table 1).** For the chemical constants, the acid indicator gives an idea about the rate of free acids. The results of this study show that this indicator remains relatively near of standards. An IA inferior to 2 proves a good conservation of essence (low quantity of free acids). On the contrary, an IA superior to 2 explains the degradation of EO (esters' hydrolyze) during its

conservation which can be damage its chemical composition [18]. The determination of the physicochemical properties is an essential step but it remains insufficient to define essential oil. Therefore, this remains primordial to determine the chromatographic profile of the aromatic essence.

Table1. ***The physicochemical indexes of Adenocarpus bacquei's essential oil.***

Property	Values
Density at 20° C	0.84 ±0.01
Acid Index	2.00 ± 0.10
Saponification index	13.24 ±0.4
Ester index	11.23 ±0.3

Values were the mean of three replicates ± Standard deviation.

3.3. Chemical composition

Analyzing the Adenocarpus bacquei's essential oil by gas chromatography paired with mass spectrum (see **Table 2**) furthermore revealed that the major compounds were the (+)-2-Bornanone (38.26%) and Eucalyptol (27.74 %) which belong to Oxygenated monoterpenes.

3.4. Phytochemical screening

The phytochemical screening's results (see **Table 3**) of Adenocarpus bacquei's leaves and stems showed the great presence of tannins catechics, anthocyanes, flavonols, catechol, Heteroside genin, C-heteroside, sterols and triterpenes, mucilage, oses and holosides, while alkaloids, antraquinones were free, O-heteroside and saponins were absent.

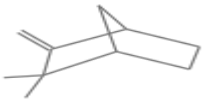
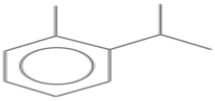
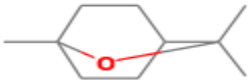
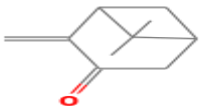
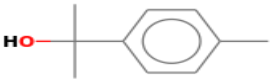
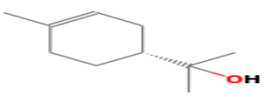
3.5. Extractions' yield of Adenocarpus bacquei

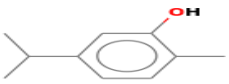
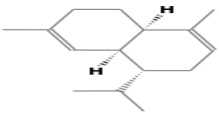
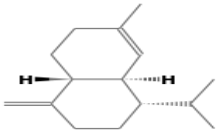
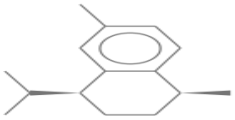
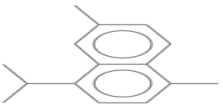
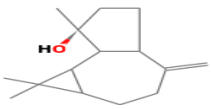
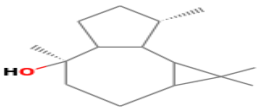
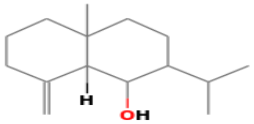
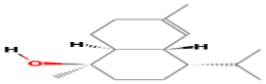
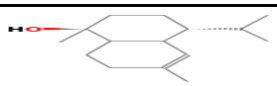
The results of yields' extractions (see **Table 4)**, using different solvents, were differ significantly ($p<0.05$).Yet for both leaves and stems of Adenocarpus bacquei showed a simultaneous increasing in extractions' yields and the solvents polarities. This result is proved by yields' values, in which water had the highest average of 17.95% followed by ethanol, ethyl acetate, dichloromethane and hexane that showed the lowest yield of extraction with 3.26%. The leaves revealed the highest yields' extraction through different solvents.

3.6. Determination of total content Phenolic (TPC) and flavonoid (TFC)

The TPC and TFC were determined by Folin-Ciocalteu's method and obeyed Olajire A and Azeez [12] protocol respectively. The results were expressed by mg GAE/g of crude extract for TPC and the TFC values were expressed by mg QE/g of crude extract. The results of Adenocarpus bacquei's crudes extracts from leaves and stems (see **Table 6**) revealed a huge distinction between the plant parts' of phenolic and flavonoid content, this distinction appears due to the richness of some and the poverty of others and that could be assigned to the solvents used during extraction. [19-21].

Table 2. *The chemical composition of Adenocarpus bacquei's essential oil.*

TR	Percentage	Nomenclature	Chemical structure	Chemical Formula
Monoterpenes Hydrocarbons:				
7.84	0.24	α-Pinene		$C_{10}H_{16}$
8.24	10.72	Camphene		$C_{10}H_{16}$
9.16	1.43	β-Pinene		$C_{10}H_{16}$
10.78	2.47	O-Cymene		$C_{10}H_{14}$
Oxygenated monoterpenes:				
11.06	27.74	Eucalyptol		$C_{10}H_{18}O$
14.58	38.26	(+)-2-Bornanone		$C_{10}H_{16}O$
14.65	1.01	Isopinocarveol		$C_{10}H_{16}O$
14.90	0.26	E-Verbenol		$C_{10}H_{16}O$
15.14	0.48	Pinocarvone		$C_{10}H_{14}O$
15.58	2.12	Isoborneol		$C_{10}H_{18}O$
16.13	0.17	p-Cymen-8-ol		$C_{10}H_{14}O$
16.23	1.15	Myrtenal		$C_{10}H_{14}O$
16.48	0.79	α-Terpineol		$C_{10}H_{18}O$

20.39	0.38	Carvacrol		$C_{10}H_{14}O$
Sesquiterpenes :				
26.03	0.24	γ-Muurolene		$C_{15}H_{24}$
26.75	0.25	α-Muurolene		$C_{15}H_{24}$
27.08	1.74	γ-Cadinene		$C_{15}H_{24}$
27.19	0.64	(-)-Calamenene		$C_{15}H_{22}$
31.24	1.87	Cadalene		$C_{15}H_{18}$
Oxygenated Sesquiterpenes :				
28.63	0.65	(+)-Spatulenol		$C_{15}H_{24}O$
29.10	0.70	(+)-Viridiflorol		$C_{15}H_{26}O$
30.16	0.73	Junenol		$C_{15}H_{26}O$
30.47	1.61	τ-Cadinol		$C_{15}H_{26}O$
30.60	0.61	β-Eudesmol		$C_{15}H_{26}O$
30.80	0.8	α-Cadinol		$C_{15}H_{26}O$

Total of compounds :97.06%

- Monoterpenes Hydrocarbons' Total: 14.86%
- Oxygenated monoterpenes' Total : 72.36%
- Sesquiterpenes' Total : 4.74%
- Oxygenated Sesquiterpenes :5.1%

TR: Time of Retention.

Table 3. ***Results of phytochemical screening of Adenocarpus bacquei's leaves and stems.***

Family	Phytoconstituent		Test performed	Leaves	Stems
Azote compound	Alkaloids		Mayer's Test	--	--
			Wanger's Test	--	--
Polyphenol	Tannins Catechics		Stiansy reaction	+++	+++
	Tannins Gallics		Lead acetate	--	--
	Anthocyanes			++	++
	Flavonols		Cyanide Reaction	+++	+++
	Catechol		Cyanide reaction without Magnesium	++	++
Anthrancenics					
	Antraquinons free		Borntrager's Test	--	--
	Antraquinons combined	O-heteroside	Modified Borntrager's	--	--
		Heteroside Genins		++	++
		C-Heteroside		++	++
Sterols and Triterpenes			Liberman-burchard	++	++
Saponins			Foam index :positive >100	--	--
Mucilage			Alcohol 95%	++	++
Oses and holosides			Alcohol saturated with Thymol	+	+

High concentration (+++); moderate concentration (++); low concentration (+); absence (--)

Between each concentration of the TPC and the TFC of all the crudes extracts a significant difference was shown ($p<0.05$).Those results proved that Adenocarpus bacquei is rich with phenolic and flavonoid compounds , precisely in its leaves that contained more phenolic concentration (347.905±1.05 to 64.11±0.45 mg of GAE/g of crude extract) than stems (324.537±1.07 to 98.958±0.41 mg of GAE/g of crude extract), also flavonoid appeared in high quantities in leaves (230.087±1.17 to 29.435±0.36 mg of QE /g of crude extract) contrary to stems which had less concentrations (200.956 ±0.93 to 51.174±0.25 mg of QE /g of crude extract). According to the results, water and ethanol had

respectively the greatest capabilities to extract total phenolic compounds by cause of owning the two highest polarities, followed by ethyl acetate dichloromethane; while hexane showed both the lowest capacity to extract phenolic compounds and the lowest polarity. It may be concluded that crude extract's affluence of phenolic compounds increases once the polarity of extraction solvents used increases too. Several studies such as [3, 22] approve that the phenolic compounds' extraction from plant's parts is more efficient with the solvent higher polar [23,24]. These crudes extracts revealed different aspects that were resumed below (see **Table 5**):

Table 4. ***Yields (%) of crudes extractions of Adenocarpus bacquei's different parts***

	Yields				
	Water	Ethanol	Ethyl acetate	Dichloromethane	Hexane
Leaves	20.85±0.3	18.5± 0.79	12.08 ± 0.04	8.58 ± 0.89	3.58± 0.06
Stems	15.05± 0.2	12.36 ± 0.15	10.63 ± 0.08	6.76± 0.33	2.94± 0.08
Means yields %	17.95	15.43	11.35	7.67	3.26

Mean value ± standard deviation of three replicates are mentioned. Values are significantly different ($p<0.05$)

Table 5. ***The aspects of crude extraction***

Solvent	**Leaves**	**Stems**
Hexane	Pasty	Powder
Dichloromethane	Solid	Pasty
Ethyl-Acetate	Powder	Powder
Ethanol	Solid	Pasty
Water	Powder	Solid

Table 6. ***Total phenolic and flavonoid contents of Adenocarpus bacquei's leaves and stems extracts.***

Bioactive compounds	**Water**	**Ethanol**	**Ethyl Acetate**	**Dichloromethane**	**Hexane**
Plant's parts					
TPC (mg GAE/g) Leaves	347.90 ±1.05	241.16 ±1.43	192.43 ±0.81	96.011±0.43	64.11±0.45
Stems	324.63 ±1.07	292.85 ±0.94	152.22 ±0.62	106.91±0.33	98.95 ±0.41
TFC (mg QE/g) Leaves	230.087±1.17	165.304±0.93	94.652 ±1.09	71.391±0.53	29.435±0.36
Stems	200.956±0.93	160.522±1.17	81.174 ±0.65	68.565±0.81	51.174±0.25

Mean value ± standard deviation of three replicates are mentioned. Values are significantly different ($p<0.05$)

3.7. Antioxidant activities of Adenocarpus baquei

Total antioxidant activity (TAC)

The valuation of total antioxidant activity of Adenocarpus bacquei's crudes extractions from leaves and stems (see **Table 7**) using the phosphomolybdenum process are expressed in mg AA/g of crude extract, and show a significant difference ($p <0.05$). The aqueous extract of Adenocarpus bacquei showed the highest TAC (the leaves with 412.44 ± 0.82 and stems 402.07±0.66 mg AA/g). Plainly, the greatest TAC is noted for aqueous extracts followed respectively

by ethanolic, ethyl acetate and dichloromethane crudes extracts' leaves and stems, while hexane crudes extracts revealed the lowest TAC (leaves with 173.56± 0.25 and stems with 183.18±0.57mg AA/g).

The DPPH free radical scavenging activity

Adenocarpus bacquei's antioxidant activity was measured by scavenging free radical DPPH assay, according to results (see **Table 7**) the aqueous leaves' extract shows the strongest radical scavenging activity [25] which is nearly similar to quercetin standard (see **Table 8**) with a significant difference ($p<0.05$), even the crude extracts show the same significant difference, whilst the weaker antioxidant activity was revealed by the stems' hexane extract.

Table 7. ***Total antioxidant capacity (TAC), Inhibitory concentration (IC_{50}), Efficient concentration (EC_{50}) and Antiradical-power (ARP) of the extracts from Adenocarpus bacquei's leaves and stems:***

Bioactive Compounds		**Water**	**Ethanol**	**Ethyl-Acetate**	**Dichloromethane**	**Hexane**
	Plant's part					
TAC (mg AA/g)	Leaves	412.44±0.82	289.85±1.15	280.22±0.25	200.09±0.25	173.56±0.25
	Stems	402.07±0.66	320.59±0.94	249.85±0.63	234.89±0.90	183.18±0.57
IC_{50} (mg/mL)	Leaves	0.18±0.00	0.57±0.00	0.81±0.00	1.16±0.00	1.53±0.01
	Stems	0.35±0.00	0.48±0.00	1.19±0.01	1.29±0.00	8.82±0.05
EC_{50}(mg/ mg_{DPPH})	Leaves	7.66±0.02	24.07±0.01	33.88±0.15	48.54±0.02	56.27±0.15
	Stems	14.87±0.01	20.32±0.02	49.67±0.15	54.05±0.07	367.5±0.21
ARP	Leaves	13.04±0.12	4.15±0.05	2.95±0.03	2.06±0.00	1.77±0.03
	Stems	6.72±0.05	4.92±0.04	2.01±0.01	1.85±0.00	0.27±0.01

Mean value ± standard deviation of three replicates are mentioned. Values are significantly different ($p<0.05$)

Table 8. ***Total antioxidant capacity (TAC), Inhibitory concentration (IC_{50}), Efficient concentration (EC_{50}) and Antiradical-power (ARP) of Standard quercetin.***

	Standard Quercitin
IC_{50} (mg/mL)	0.10 ±0.00
EC_{50} (mg/mg_{DPPH})	0.04 ± 0.00
ARP	24.03 ±0.00

Table 9. ***Total antioxidant capacity (TAC), Inhibitory concentration (IC_{50}),Efficient concentration (EC_{50}) and Antiradical-power (ARP) of Adenocarpus bacquei's essential oil***

Essential oil	**TAC(mg AA/g)**	**IC_{50}(mg/mL)**	**EC_{50}(mg/mgDPPH)**	**ARP**
	708.37 ± 0.94	0.77 ± 0.00	32.20 ± 0.02	3.10 ± 0.12

Values were the mean of three replicates ± Standard deviation.

Antioxidant activity of essential oil

The phosphomolybdenum process and scavenging free radical DPPH assays were used to estimate the antioxidant activities of Adenocarpus bacquei's essential oil (see **Table 9**). Its TAC was stronger than crude extracts' TAC, while its radical scavenging capacity presents less than 8% of the quercetin standard capacity.

3.8. Correlation

The addiction of antioxidant activities was calculated, during this study, through the DPPH free radical scavenging and the TAC assays that showed a positive correlation with the TPC and the TFC of Adenocarpus bacquei's crudes extracts. The results exhibit a positive correlation of TPC with TAC (R^2 = 0.892) and the DPPH free radical scavenging (R^2 = 0.7303) (see **Figure 1**), yet the TFC (see **Figure 2**) correlates positively to TAC (R^2 = 0.9225) and the DPPH free radical scavenging (R^2 = 0.7913). Evenly, the TAC and the DPPH free radical scavenging present a positive linearly correlation with R^2 = 0.7842 (see **Figure 3**). As a result of this work, different part of an aromatic plant's phenolic content may be the major contributor of the antioxidant activities, which is similar to previous studies [3], [26].

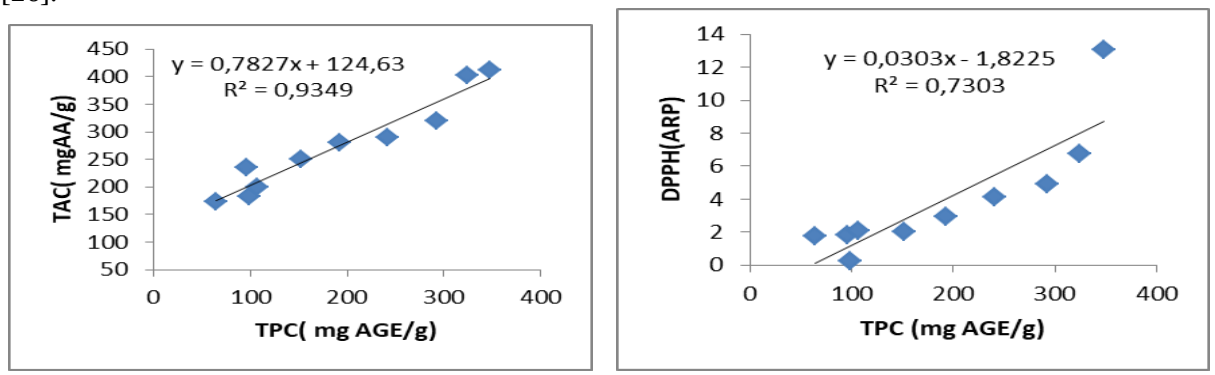


Figure 1. Linear dependency between antioxidant activity TPC and DPPH assay.

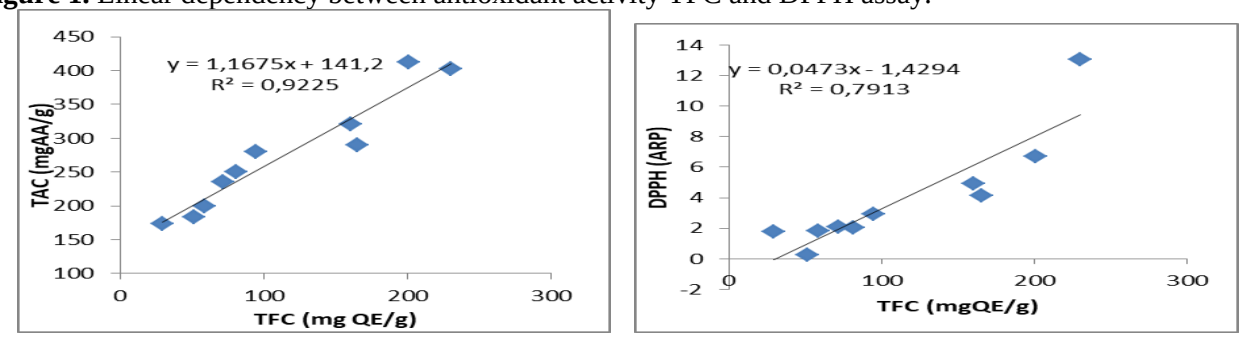


Figure 2. Linear dependency between antioxidant activity TFC and DPPH assay

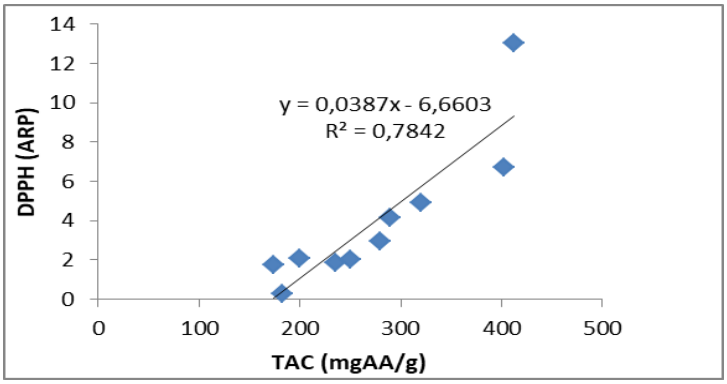


Figure 3. Linear dependency between antioxidant activity TAC and DPPH scavenging activity

Conclusion

This study aimed to valorize the Adenocarpus bacquei's extracts and essential oil. The quality control of Adenocarpus bacquei's essential oil using physicochemical characteristics served to highlight this essential oil's quality, which is distinguished by low values of acidity and ester indexes. Thus, the results of this work showed high values of acidity and ester indexes, evenly the essential oil revealed the greatest antioxidant capacities. Furthermore, using different polar solvents influence significantly ($p < 0.05$) total phenolic compounds, total flavonoid content and antioxidant activities of different parts of Adenocarpus bacquei's crudes extracts. These results indicated that the leaves' aqueous extract of Adenocarpus bacquei had the uppermost total phenolic, flavonoid concentrations and the greatest antioxidant capacities by the TAC and the DPPH assays. In addition, the TFC and TPC correlated significantly with the antioxidant activities measured by the DPPH radical and TAC assays. Therefore, these methods are suitable to evaluate the antioxidant capacities of Adenocarpus bacquei. The results suggested that the Adenocarpus bacquei's essential oil and crude extracts could be considered a potential natural source of antioxidants which prohibit oxidative stress' development.

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