Research Article

Preliminary Phytochemical Analysis and *in Vitro* Antioxidant Activity of *Boscia arabica* Leaves

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Received: 18-02-2021; **Revised:** 25-04-2021; **Accepted:** 30-04-2021; **Published on:** 15-05-2021.

ABSTRACT

Boscia arabica (Capparaceae), popularly known as "Sarh", is a plant utilized in Yemen in traditional medicine for treatment of many ailments, but very few studies have been carried out on this plant. The *Boscia arabica* leaves were selected to phytochemical analysis and evaluate antioxidant activity. Physico-chemical parameters of leaves powder were established and are within the acceptable ranges. The leaves powder was treated with various reagents and the mixture was observed under day light and under UV light to see the type of fluorescence. Different fluorescence colours were observed. Chemical analysis was carried out to determine the major chemical classes, phytoconstituents like carbohydrates, saponins, flavonoids, triterpenes, sterols and alkaloids were identified in studied extracts. The presence of phytoconstituents was further confirmed by thin layer chromatography and Rf values of developed spots were calculated with color intensity. Antioxidant activity of *Boscia arabica* leaf extracts was carried out via DPPH staining technique. The result of dot-blot assay showed yellow-coloured spots when stained with DPPH solution. The free radical scavenging activity of 80% methanol extract and it's fractions was determined and antioxidants activity ranged from (14.81±3.22) for methanol 80% extract to (85.31±3.12) for n-butanol fraction. Ethyl acetate (84.30±2.44) and n-butanol (85.31±3.12) are exhibited high free radical scavenging activity when compared with quercetin **(**99.10±4.22). The results obtained in this study support the use of *Boscia arabica* in traditional medicine, which may be a promising source for the development of natural antioxidants.

Keywords: *Boscia arabica*, Yemen, phytochemical, antioxidant.

QUICK RESPONSE CODE →

DOI:
10.47583/ijpsrr.2021.v68i01.022

DOI link: http://dx.doi.org/10.47583/ijpsrr.2021.v68i01.022

INTRODUCTION

The study of medicinal plants is one of the most important topics of interest to researchers in Yemen. The traditional medicinal methods still play a vital role in the prevention, diagnosis and treatment of illness in Yemen, and most of the medicinal substances are plant-based, a reflection of the country's rich vegetation[1]. To date, only few medicinal plants have been studied[2], Therefore, there is a need to evaluate phytoconstituents obtained from traditional medicines, based on various phytochemical screening and pharmacological and analytical methods[3]. According to World Health Organization (WHO) pharmacognostic parameters are necessary for confirmation of the identity and determination of quality and purity of a crude drug used in traditional medicine[4]. The plant kingdom is a wide range of natural antioxidants. In the category of secondary plant metabolites, antioxidant phenolics are commonly found in plants and they have indicated providing a defense against oxidative stress[5,6] .

The genus Boscia (Capparaceae) contains more than 37 species distributed mainly in Africa, excluding one species found in southern Arabia[7]. *Boscia arabica* Pestalozii is endemic to Arabian Peninsula and is found only in Yemen and Saudi Arabia[8]. It is used by Yemeni people in traditional medicine for treatment of many diseases including kidney disease. Up to date, no pharmacognostic, phytochemical or pharmacological studies have been reported for this plant. The leaves of *Boscia arabica* were selected with the aim of phytochemical analysis and evaluation of antioxidant activity. The present study is an essential step and requires long-term phytochemical and pharmacological studies.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Boscia arabica* pestalozzi were collected in July 2018 from Abyan, Republic of Yemen, dried in the shaded area and then manually grinded and stored at room temperature for further analysis. The plant sample was identified by a taxonomist, Associate Professor Othman S. Alhawshabi, of the faculty of Science, University of Aden, Yemen.

Physicochemical analysis

The physicochemical parameters such as loss on drying, total ash, acid insoluble ash, water soluble ash, water soluble extractive and ethanol soluble extractive were carried out in dried leaves powder according to standard methods[9,10].

Fluorescence analysis of powdered drug

A fine powder of studied leaves was placed on a grease free clean microscopic slide and added 1-2 drops of the freshly prepared reagent solutions mixed properly and waited for 1-2 minutes. Then the slide was viewed in day light and inside the UV viewer chamber short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in different radiations were recorded[11].

Preparation of extracts

The dried powdered leaves (50 gm) were defatted with petroleum ether (boiling point 60-80 ^{0}C) in soxhlet extractor. The marc left after petroleum ether extraction was dried completely in hot air oven below 50°C and then packed well in soxhlet apparatus and extracted with 80% methanol (80-90°C), until the extraction was completed. The marc after that was dried and extracted with sterilized water. Resulting extracts in different solvents were evaporated and concentrated to dryness using the rotary evaporator at 50°C and the percentage yield in terms of air dried material was calculated. The dry extracts were used for further analysis[12,13].

Fractionation of 80% methanol extract

Fractionation of 80% methanol extract was carried out with different organic solvents. Methanol (80%) extract was suspended in water, extracted successively with chloroform, ethyl acetate and n butanol (6×300 ml each) and then resulting solutions were concentrated to provide chloroform, ethyl acetate, n butanol and water residue fractions. The rest of methanolic extract (20%) was used for further phytochemical analysis[13].

Qualitative phytochemical analysis

Preliminary analysis of 80% methanol and water extracts was carried out to identify the presence of various phytoconstituents by employing standard protocols[13,14].

Thin layer chromatography studies

Thin Layer Chromatography of prepared extracts was performed to determine Rf values. Various solvent systems were tested to obtain best results. TLC plates were first viewed in day light then in UV chamber before keeping in iodine chamber and Rf of all were noted. Different solvent systems were found to be effective to get maximum no. of spots for various extracts[15,16].

Antioxidant studies

Rapid screening of antioxidant by dot-blot and DPPH staining

Each diluted sample of the extracts/fractions was carefully loaded onto a 20 cm × 20 cm TLC layer (silica gel 60 F254; Merck) and allowed to dry (3 min). Drops of each sample were loaded, in order of decreasing concentration (0.5, 0.25 and 0.125 mg/ mL), along the row. The staining of the silica plate was based on the procedure. The sheet bearing the dry spots was placed upside down for 10 s in a 0.05% 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution. Then the excess of solution was removed with a tissue paper and the layer was dried with a hair-dryer blowing cold air. Stained silica layer revealed a purple background with yellow spots at the location where radical-scavenger capacity presented. The intensity of the yellow color depends upon the amount and nature of radical scavenger present in the sample[17].

DPPH Radical Scavenging Activity

The scavenging activity for DPPH free radicals was measured according to the procedure described by the Braca et al., with some modifications[18]. In the assay, the DPPH reagent was prepared by dissolving the 0.04 g DPPH in 100ml of methanol. From this stock 3 ml of DPPH solution in methanol and 0.1 ml of plant extract at various concentrations or fractions (50-250 µg/ml) were mixed. The mixtures vortexed and allowed for 30 min to reach a steady state at room temperature. Decolonization of DPPH was determined by measuring the absorbance at 517 nm. A control was prepared using 0.1 ml of respective vehicle in the place of plant extract.

The percentage inhibition activity was calculated as extract/ascorbic acid.

Inhibitory ratio = (A0 - A1) / A_0 ×100

Where, A0 is the absorbance of control (quercetin); A1 is the absorbance of the plant extract.

IC50 value

Inhibition Concentration (IC50) parameter was used[19], for the interpretation of the results from DPPH method. The discoloration of sample was plotted against the sample concentration in order to calculate the IC50 value. It is defined as the amount of sample necessary to decrease the absorbance of DPPH by 50 %.

RESULTS AND DISCUSSION

Physicochemical analysis

In accordance with WHO guidelines, it is important to evaluate the physicochemical and phytochemical characters of crude drug for developing standardized quality control profile of herbal medicine[10,20]. In the present study, various parameters like ash content, solvent extractive values, moisture, were estimated for *Boscia arabica* leaves and are within the acceptable ranges. The results obtained were described in Table 1. Ash values are important quantitative standards used to find out quality, identity and purity of crude drugs especially in the powder form[21,22]. The extractive value gives an idea about the nature of the chemical constituents present in a crude drug and furthermore assist in the evaluation of definite constituents soluble in a particular solvent[23,24]. Excess of water content in plant materials results in microbial growth and deterioration, so determination of moisture content is another important parameter that should be evaluated for plant drugs[25]. Dried herbal drug is expected

to have a long shelf-life with reduced chance of microbial growth due to its relatively low moisture content[26].

Fluorescence analysis of powdered drug

Fluorescence study powdered drug under ultra violet light is very distinctive and helpful in establishing the purity of the drug[27]. The fluorescence analysis of the powder drug was done. The powder of studied leaves was treated with various reagents and the mixture was observed under day light and under UV light to see the type of fluorescence. The results are given in Table 2.

Table 1: Physico-chemical parameter of leaves of *Boscia arabica* leaves

Physicochemical parameters Leaf (%w/w)	Physicochemical parameters Leaf (%w/w)
Ash values:	
1) Total ash	5.10 ±0.06
2) Acid insoluble ash	1.51 ±0.02
3) Water soluble ash	3.57 ±0.04
Extractive value:	
1) Water soluble	42.50 ±0.05
2) Ethanol soluble	10.10 ±0.05
Moisture content Loss on drying at 110ºC	6.66±0.04

Table 2: Fluorescence analysis of powdered *Boscia arabica* leaves.

S. No.	Treatments	Observations	
		Day light	Long UV
1	Powder + 1N NaOH	Brown	greenish yellow
2	Powder + 1N H2SO4	Yellow	Deep yellow
3	Powder + 50% N HNO3	Brownish red	Brownish yellow
4	Powder + conc.HNO3	Reddish brown	Brown
5	Powder + dil HNO3 10%	Brown	Greenish yellow
6	Powder + Ammonia	yellow	Greenish yellow
7	Powder + Acetate	Black	Red
8	Powder + 50% Iodine	Brownish yellow	Greenish yellow
9	Powder + 5% FeCl3	Black	Black
10	Powder + Methanol	Greenish	Red
11	Powder + water	Brownish yellow	Brown

Preliminary phytochemical screening

The percentage yield of petroleum ether, methanol 80% and water extracts of the leaves powder of *Boscia arabica* were determined and the result shown in the Table 3. Phytochemical screening of the methanol 80% and water extracts was done and results were represented in Table 4. Phytoconstituets like carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols and alkaloids were identified in studied extracts. Identification of the different classes of phytochemical constituents of the plant is an important parameter which gives an indication of the pharmacological active metabolites present in that plant[28]. Secondary metabolites of plants are reported to have many biological and therapeutic properties[29-31]. The phenolic compounds have been reported to have anti-oxidants property due to their conjugated ring structures and the presence of hydroxyl groups[32,33], also they have been reported to exhibit cardioprotective, hepatoprotective, anti-allergenic, anti-inflammatory, antimicrobial, antithrombotic, anti-atherogenic, and vasodilatory effects[34,35].

Table 3: The percentage yield of petroleum ether, methanol and water extracts of the leaves of *Boscia arabica*

S. No.	Solvent	Weight of plant material (gm)	Percentage of Yield (%)	Colors of extracts
1	Petroleum Ether	50	1.50	Green
2	Methanol 80%	50	17.00	Greenish brown
3	Water	50	40.60	Reddish brown

Table 4: Results of phytochemical screenings of successive extracts of the leaves of *Boscia arabica*.

Phytochemical Screening		Methanol 80% extract	Water extract
Alkaloids	Wagner's test	+++	++
	Mayer's test	+++	-
	Dragendorff's reagent	+++	-
Polyphenols	Ferric chloride test	++	+++
Flavonoids	Shinoda test	++	++
	NaOH Test	+++	+++
	Lead acetate test	++	++
	Aluminium solution test	++	++
Saponins	Foam test	++	+++
Sterols/ Triterpenes	Salkowski test	+++	+++
	Liebermann-Burchard test	+++	+++
Carbohydrates	Molisch's test	++	++
	Fehling's test	++	+
Tannins	Ferric chloride test	++	+++
	Gelatin test	-	-

+++ = Most intense, ++ = moderately intense, + = Least intense, - = absent.

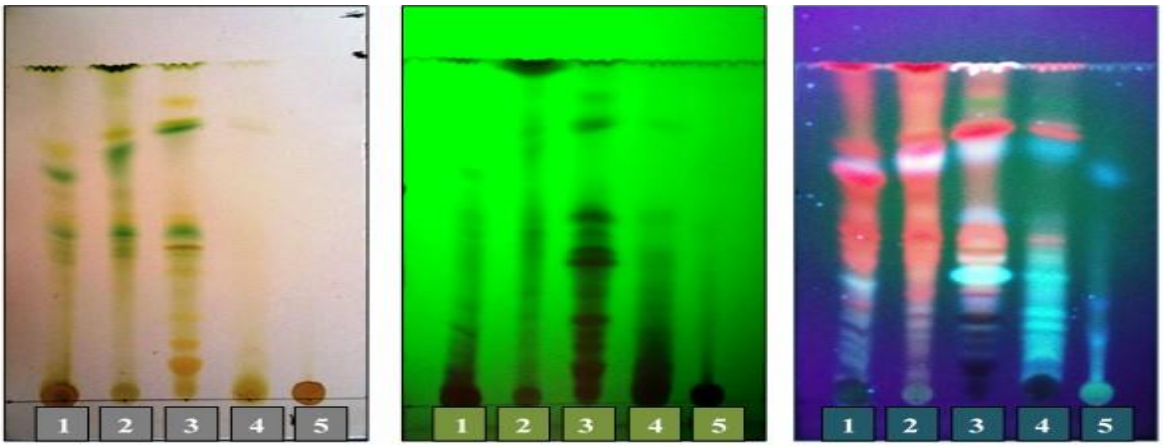
Thin layer chromatography

TLC is still the basic tool for the separation and identification of natural compounds. It is often used to provide the first characteristic fingerprints of herbs[36]. TLC (silica gel G 60 F254 TLC plates of layer thickness 0.2mm, Allugram- Germany) of prepared methanol 80% extract and chloroform, ethyl acetate, n-butanol and water fractions was performed. The best solvent systems was Chloroform – Methanol – Formic acid (44:3.5:2.5). Photos of the plates were taken in day light and in UV chamber and *Rf* values of developed spots of different extracts were calculated as well as the colour of spots were observed, which are mentioned in Table 5 and Figure 1.

Table 5: Observations of thin layer chromatographic of methanol 80% extract and its fractions of *Boscia arabica leaves* in Chloroform – Methanol – Formic acid (44:3.5:2.5)

Extract or Fractions	No. of spots	Rf values	Colour of the spot in daily light	Colour of the spot at 254 nm	Colour of the spot at 365 nm
Methanol 80% extract	12	6.5	Light green	Quenching	Red
		6.1	Not visible		white fluorescent
		5.9	Green		Red
		4.2			Red orange
		3.8			Orange
		6.3			Orange
		3.1	Not visible		Blue fluorescent
		2.7			Orange
		2.0			Sky blue
		1.4			Sky blue
		1.0			Sky blue
		0.8			Sky blue
Chloroform fraction	11	6.5	Greenish brown	Quenching	Red
		6.1	Green		white fluorescent
		4.2	Dark		Red
		3.8	Not visible		Red

		3.6			Red orange
		3.0	Green		Red orange
		2.6	Greenish brown		Red orange
		2.0	Not visible		Blue orange
		1.4			Blue orange
		1.0			Blue orange
		0.6			Blue orange
Ethyl acetate fraction	13	6.8	Brown	Quenching-Deep	Red
		6.1	Green		white fluorescent
		4.4	Not visible		sky
		4.2			Red orange
		3.6			Orange yellow
		3.1	Green		Sky fluorescent
		2.7	Deep brown		Orange blue
		2.2	Not visible		Greenish blue
		2.0			Blue violet
		1.6			Greenish blue
		1.4			Greenish blue
		1.0	Orange brown		Greenish blue
		0.7			Blue violet
n-Butanol Fraction	8	6.7	Not visible	Not visible	Orange red
		6.1	Light brown	Dark	Blue
		4.2	Not visible	Quenching	Pink
		3.6			Sky blue
		3.1			Sky blue
		2.2			Sky blue
		1.8			Sky blue
		0.1			Sky blue
Water Residue	3	5.9	Not visible	Not visible	Sky blue
		2.7			Sky blue
		1.4			Sky blue



A B C

Figure 1: TLC plate of methanol 80% extract (1) and its fractions; Chloroform (2), Ethyl acetate (3), n-Butanol (4) and Water (5), obtained in Chloroform – Methanol – Formic acid (44:3.5:2.5) under daily light (A), UV 254 (B) and UV 365 (C).

Antioxidant studies

Medicinal plants have been used for human healthcare and represent the mainstay of traditional systems of medicine. The importance of plants as natural sources of antioxidants and free radical scavengers has been reported earlier by various researchers[35,37]. Numerous methods to evaluate the antioxidant activity of specific compounds have been described, but the most widely documented relates to the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), for fast and easy evaluation of the antioxidant activity due to its stability, reliability and the simplicity of the assay[38-40]. Compounds with antioxidant properties would change the purple color of DPPH to yellow as the radical is quenched by the antioxidant[40]. The antioxidant activity of *Boscia arabica* leaves was studied by the DPPH method.

Rapid screening of antioxidant by dot-blot and DPPH staining

The antioxidant potential activity of methanol 80% extract and its fractions was determined via eye-detected semi-quantitatively via a rapid DPPH staining-TLC technique. The dot-blot test is an easy, fast and reliable way to compare radical scavenging capacity of various plant extracts[41]. Each diluted samples were applied as a dot on a TLC layer that was then stained with DPPH solution. Quercetin was used as a positive control. The results of dot-blot assay showed yellow colored spot when stained with DPPH solution (Figure 2). All dots at concentration of 0.5mg/ml, 0.25 mg/ml, 0.125 mg/ml and 0.0625mg/ml showed high antioxidant activity when compared with control (quercetin), exception water fraction, which showed antioxidant activity only at concentration of 0.5mg/ml and 0.25 mg/ml.

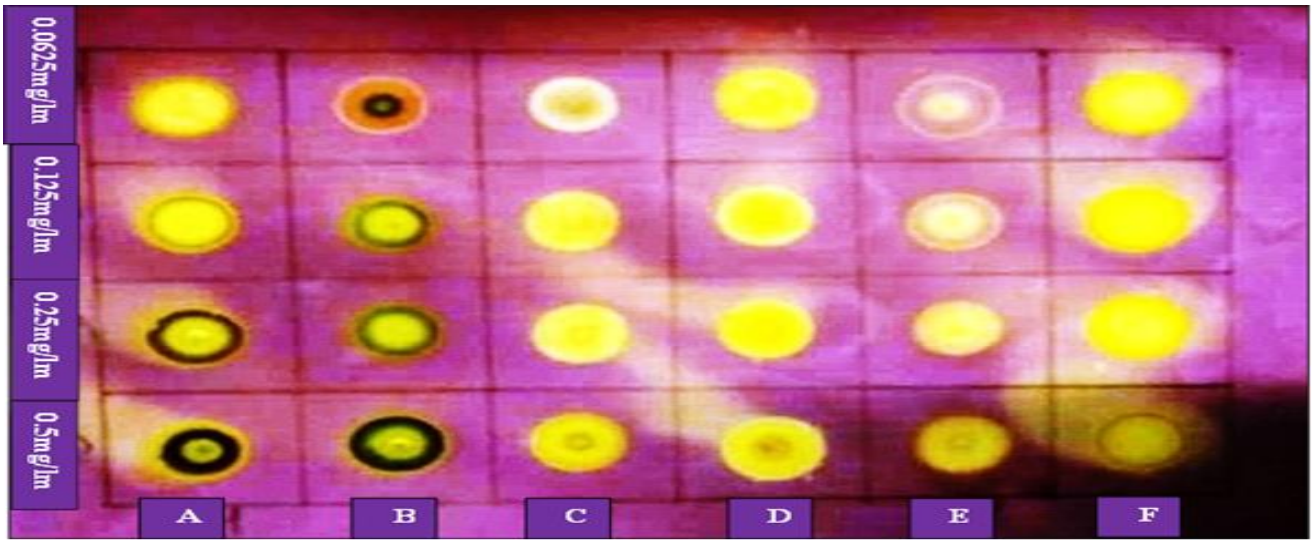


Figure 2: TLC plat of methanol 80% extract (A) and its fractions; chloroform (B), ethyl acetate (C), n-butanol (D) and water (E), and quercetin (F) on 0.5mg/ml, 0.25mg/ml, 0.125mg/ml and 0.0625mg/ml stained with DPPH.

DPPH radical scavenging activity assay

The antioxidant activity of methanol 80% extract and it's fractions (chloroform, ethyl acetate, n-butanol) of *Boscia arabica* leaves was determined. The free radical scavenging activity of 80% methanol extract and it's fractions was determined (Table 6). Antioxidants activity ranged from (1.26±4.20) for chloroform fraction to (85.31±3.12) for n-butanol fraction. Ethyl acetate (84.30±2.44) and n-butanol (85.31±3.12) are exhibited high free radical scavenging activity when compared with Quercetin (99.10±4.22).

The percentage (%) scavenging of DPPH free radical was found to be concentration dependent, i.e. concentration of the extract between 50-200 mcg/ml greatly increasing the inhibitory activity (Figure 3). Ethyl acetate and n-butanol are exhibited high free radical scavenging activity.

Table 6: The DPPH free radical scavenging activity of the methanolic 80% extract and it's fractions (chloroform, ethyl acetate, n-butanol) of aerial parts of *Boscia arabica* and Quercetin

Concentration µg/ml	Radical scavenging effect (%)				
	Quercetin	Methanol 80% extract	Chloroform fraction	Ethyl acetate fraction	n-Butanol fraction
50	93.00±2.22	14.81±3.22	1.26±4.20	45.96±3.23	62.91±3.88
100	98.00±4.12	33.14±1.24	3.67±2.41	68.60±4.01	83.61±4.18
150	98.60±3.25	46.96±3.01	7.84±3.32	83.54±3.22	85.06±3.10
200	99.10±4.22	60.88±2.11	16.84±4.20	84.30±2.44	85.31±3.12

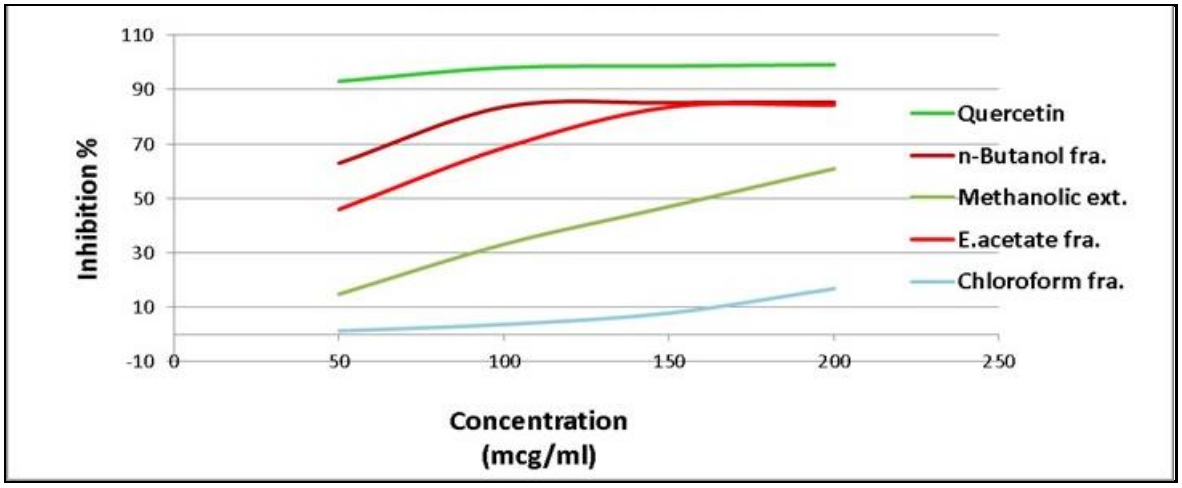


Figure 3: DPPH free radical scavenging activity of methanolic extract it's fractions and Quercetin

CONCLUSIONS

The present study indicates that the leaves of *Boscia arabica (Capparaceae)* might serve as important medicinal plant due to the presence of phytochemicals such as carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols and alkaloids, and might give approval to its local usage in the treatment of various diseases. The results of the current study establish the various physicochemical parameters, fluorescence analysis, TLC profile and antioxidant activity of *Boscia Arabica*, which is endemic to Yemen. This data may help in standardizing the plant as it is an important analytical aspect for evaluating the identity, quality and purity plant drugs. Further deep pharmacognostica and pharmacological investigation is very essential to prove the efficacy of this medicinal herb. More detailed study must be done for further isolation leading to the pure compounds and establishment pharmacological activities of this drug.

Acknowledgement: The authors are grateful to Associate Professor Othman S. Alhawshabi, of the department of Biology, of University of Aden, for his help to identify and authenticated the plant specimen.

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Source of Support: None declared.

Conflict of Interest: None declared.