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# Antioxidant activity from the aerial parts of *Anisotestrisulcus* from Yemen

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## ABSTRACT

**Background:** *Anisotestrisulcus* (Forssk.) Nees. (familyAcanthaceae) aerial part is used in folk medicine in the Arabian peninsula for treatment of hepatic conditions. It showed different activities such as antibacterial, hepatoprotective, and cytotoxicity. It is a rich source of alkaloids and is used as an antidiabetic, bronchodilator, hypotensive, and local anesthetic. To the best of our knowledge, there is no report on the phenolic constituents of *A. trisulcus*. **Objectives:** to evaluate antioxidant activity of aqueous extracts from *Anisotestrisulcus.* **Results:** Results from antioxidant activity assays showed that ethanol in DPPH radical scavenging is more active than Petroleum ether and chloroform. This study suggested that the differences of the potency of the antioxidant activity may be explained by the differences in the polyphenol and flavonoid levels. **Conclusion:** *Anisotestrisulcus* leaves possesses significant antioxidant activity.

**KEYWORDS:** *Anisotestrisulcus, Antioxidant activity, DPPH*

## INTRODUCTION

Reactive oxygen species are produced as a result of physiological processes as byproducts of energy production, for the prevention from the invasive microorganisms or as signaling and regulatory molecules [1]. The deleterious effects of the reactive species occur in the lack of the equilibrium between oxidation and antioxidant defense in the body. Oxidative stress may cause a serious damage in the body, as reactive oxygen species are known to be highly responsible from oxidation of biological molecules; such as lipids, DNA and proteins [1].

The genus Anisotescontains 23 species, which are represented by herbs, shrubs or sometimes climbers. [2,3].*Anisotestrisulcus* (Forssk.) Nees. (familyAcanthaceae) is a stiff erect shrub with a height up to 3.5 m (Figure 1). It is monopodially branched carrying opposite decussate leaves at the nodes bearing bright orange–red colored tubular flowers. [3] *A. trisulcus*is known as Almodh, Madh, Moze, and Modaid in the Kingdom of Saudi Arabia (KSA) [3]. It is used locally in several pharmaceutical forms to limit tobacco consumption and suppress appetite [4]. The plant was known to possess anti-bacterial, cytotoxic, bronchodilator, hypotensive, local anesthetic, antidiabetic, and hepato-protective activities [2, 5,6 , 7]. It is also used as treatment for all hepatic conditions including hepatitis, jaundice, gallstone, and other hepatic problems [2, 6]. Previous phytochemical study of A. trisulcus revealed the isolation and identification of several alkaloids such as 5-hydroxy vasentine, 7-hydroxy vasicine, 7-hydroxy vasicinone, anisotine, peganine, vasicinone, 5-methoxypeganine, and trisulcusine [2, 8 ,9]. In continuation of our investigation, we report herein the isolation

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and structural elucidation of one new benzoyl flavonol: 7,8,30-trihydroxy-5-methoxy-40-benzoyl flavonol (5), along with eight known compounds: a-amyrin (1), b-sitosterol (2), stigmasterol (3), (2S,3S,4R)-2[(20R)-20-hydroxytetracosanoylamino]-octadecane-1,3,4-triol (4,allopateuletin (6), veratric acid (7), vanillic acid (8), and b-sitosterol-3-O-b-D-glucopyranoside (9) from *A. trisulcus*aerial parts . Their structures were established by various spectroscopic methods as well as comparison withauthentic samples. The total methanolic (MeOH) extract and different fractions were evaluated for their antioxidant and anti-inflammatory activities.

In this study, the antioxidant activity of *trisulcus* (Forssk.)Nees. (familyAcanthaceae) were evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay. DPPH is a free radical which has maximum UV absorption at 515 nm. The antioxidant capacity of the sample was determined by measuring the decrease in absorbance of DPPH after reduction.

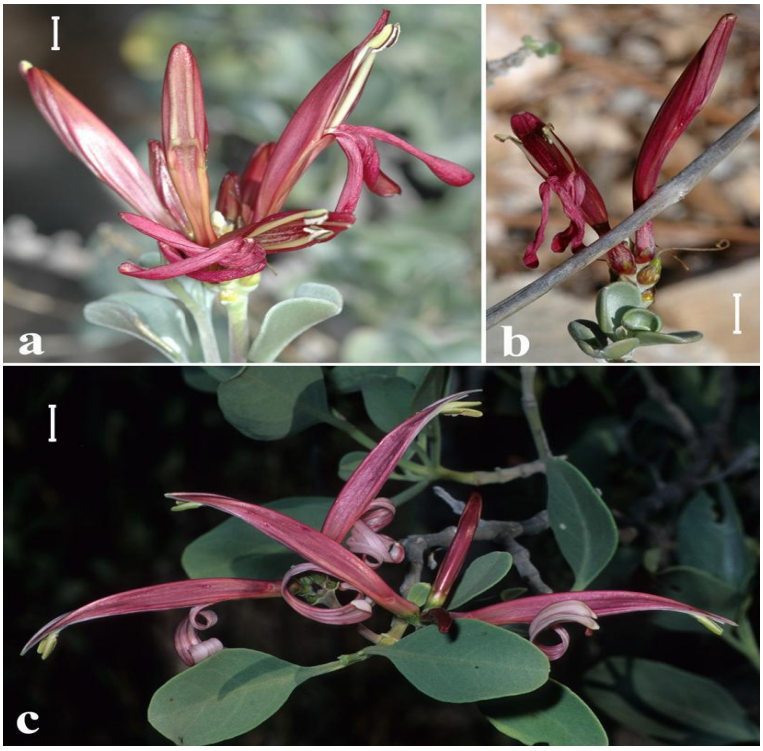


**Fig. 1:** *Anisotestrisulcus*

## MATERIAL AND METHODS

*Plant material:*

The aerial parts of *Anisotestrisulcus* was collected in March 2016 during the flowering stage in the Hajjah area of Yemen. The plant was identified and authenticated by Dr. Prof.Abdellah Amine (College of Agriculture, Sana'a University).

*2.2. Chemicals:*

All chemical reagents were of analytical grade and all solvent were purchased from Sigma-Aldrich St. Louis, MO, USA).

*Preparation of the Extracts:*

Fifty grams leaves from plant was individually extracted with 300 ml of 95% methanol, Ethanol and diethyl ether (separately) in an ultrasonic device at room temperature, The extracts were filtered and the residues were re-percolated for three times and was eliminated from the solvent using a rotary evaporator and sample was preserved at 4 ˚C until used.

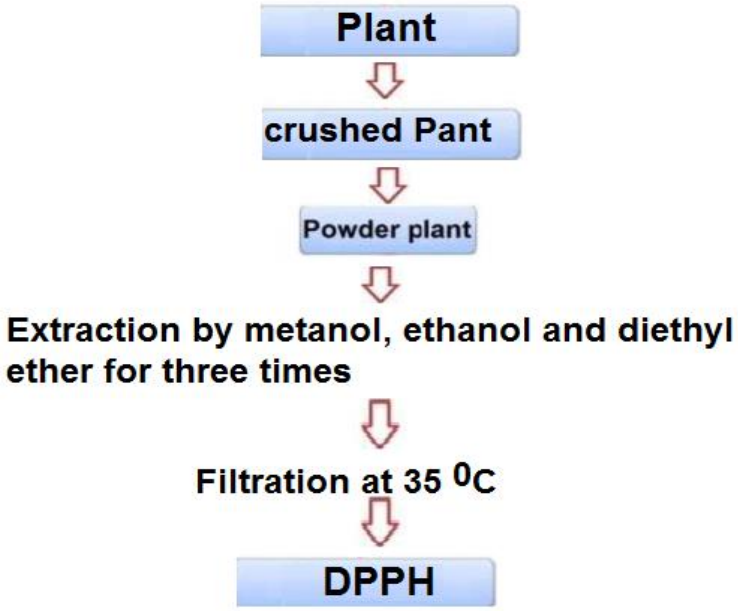


**Fig. 2:** Experiment flowchart for extract of*Anisotestrisulcus*plant

*Antioxidant activity:*

The antioxidant activity was determined as previously outlinedby the decrease in the absorption of each concentration (0.25,0.5, and 1 mg/mL) in DPPH solution (4 mg was dissolved inHPLC MeOH 50 mL to obtain a concentration of 80 µg/mL)monitored at 517 nm using a spectrophotometer[10,11,12]. Theabsorbance of DPPH in MeOH was measured after 2 min. Theantioxidant activity of total MeOH extract and different fractionswas measured in relation to TBHQ (as a reference antioxidant)set as 100% antioxidant activity. Determinations wereperformed in triplicate. The antioxidant activity was calculatedusing the following equation:

Antioxidant activity: $100 \times 1 - \frac{\text{absorbance with compound}}{\text{absorbance of the blank}}$

*Statistical Analysis:*

The experiments were conducted at least in triplicate. Analysis of variance (ANOVA, one-way) was used to separate means at a 0.05 significant level (SPSS Version 13.0 for Windows, SPSS Inc., Chicago, IL).

## RESULTS AND DISCUSSION

The present study show little antioxidant activity less than 50%. in extraction by ethanol show antioxidant activity of 13+-0.05 % less than 50% , extraction by methanol show negative result means zero antioxidant activity , also extraction by chloroform give result of 7+-0.02 also less than we need to be active . propylgalate is the stander that we use as antioxidant stander .

In previous study[13].The antioxidant activity for *Anisotestrisulcus* in agreement to the present study using DPPH radical scavenging assay. DPPH show negative result in three different concentration sample (0.25, 0.5 and 1.00)means low antioxidant percent and so not specific [13]. In bio- medicinal research laboratory , India, Jigani industrial area for evaluation of antioxidant activity of medicinal plant constitute *Anisotestrisulcus* show positive result (0.65+-0.11) using DPPH radical scavenging assay appear high pecent more than 50% and so high specific to use.[14]. the previous study of Vidyanagar K Arad , Maharashtra, India -415109, for evaluation of antioxidant activity show it is rich source of poly phenol which led to the conclusion that high soluble phenolic in extract taken to strong free radical scavenging activity [15].The present study in agreement with [13].and different to other research this referentially due to different in climate of previous study between India , Sudan and Cairo areas .Sudan and Cairo in equator area and India above equator area.

In this study, radical scavenging activity was determined for the selected extracts of *Anisotestrisulcus*plant. Being a stable free radical, the DPPH assay is a simple and rapid method frequently used to evaluate the ability of antioxidants to scavenge free radicals. As shown in**(Fig.3)**, all the sample extracts exhibited significant dose dependent inhibition of DPPH activity that rapidly increase from 1.5 to 6.5 mg/ml. This showed that the young leaves exhibit a strong scavenging activity and it has been reported that phytochemicals especially plant phenolics constitute a major group of compounds that act as primary antioxidant [16]. Medicinal plants used in

traditional medicine and healing are among natural antioxidant sources. Plants arerich sources of phenolic compounds such asflavonoids, tannins and anthocyanin's, which are the most importantnatural antioxidants [17].Plants provide flavors, fragrances and traditional medicines as part of everydaylife.Approximately 3000 plant species contain therapeutic activities, of which only 300 are commercially important[18].

**Table 1:** Antioxidant activity (DPPH) of *Anisotestrisulcus* extracts with one concentration.

| NO | Sample Code | DPPH scavenging test |
|---|---|---|
| 1 | Ethanol | 90±0.05 |
| 2 | Petroleum ether | inactive |
| 3 | Chloroform | 70±0.02 |
| 4 | TBHQ | 91±0.02 |

The values are means of three independent analyses.

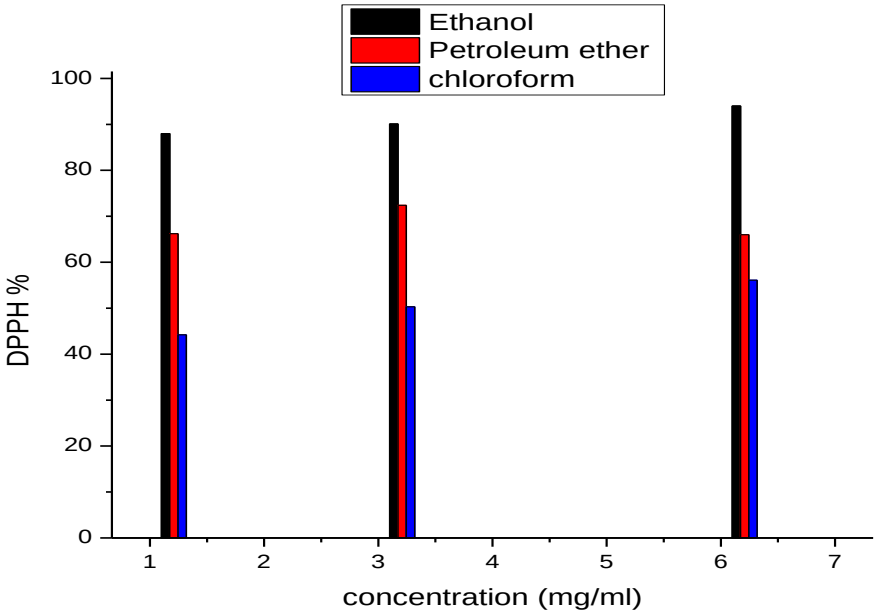


**Fig. 3:** DPPH radical scavenging activity of *P. inuloides*extractswith different concentrations. Data are expressed as mean ± SD (standard deviation) (n = 3).

*Conclusion:*

The results obtained in this study clearly showed that ethanol extracts from the leaves of *Anisotestrisulcus*possess antioxidant activity which may be caused by the high content of polyphenols and flavonoids. The very strong antioxidant activity of

*Anisotestrisulcus*suggests that the extracts obtained by polar solvents from the leaves could be used as an effective natural source of antioxidant and food additives.

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