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ARTICLE

Evaluation of antioxidant properties of *Tectaria paradoxa* (Fée.) Sledge and *Bolbitis appendiculata* (Willd.) K. Iwats

Venkatasamy Manivannam[1], Marimuthu Johnson[1], Ana Carolina Araújo[2], Priscilla Freitas[2] & Henrique Coutinho[2]

1 Centre for Plant Biotechnology, Department of Botany, St. Xavier's College (Autonomous), Palayamkottai-627 002, Tamil Nadu, India

2 Laboratory of Microbiology and Molecular Biology-LMBM, Regional University of Cariri-URCA, Crato, Ceara, Brazil

Correspondence
H.D.M. Coutinho
E-mail: hdmcoutinho@gmail.com

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Resumen

Evaluación de las propiedades antioxidantes de Tectaria paradoxa *(Fee.) Sledge y* Bolbitis appendiculata *(Willd.) K. Iwats*

El presente estudio tuvo como objetivo determinar las capacidades de captación de radicales libres de *T. paradoxa* (TP) y *B. appendiculata* (BA) utilizando el ensayo DPPH, SOD, fosfomolibeno y ABTs. El mejore valor de CI_{50} en los ensayos de DPPH de para extractos de TP fue el metanólico (102,25 μg / mL) y para los extractos de BA, la acetona (121,06 μg/mL). Entre los diversos extractos probados, los extractos metanólicos de BA y TP mostraron la mayor actividad antioxidante. La capacidad de captación de radicales libres de SOD de los extractos de TP fue CI_{50}= 123,46 μg/ml con éter de petróleo y de los extractos de BA, CI_{50}= 108,7 μg/ml con acetona. La capacidad de eliminación de radicales libres de los extractos de BA y TP fue acetona (CI_{50} = 76,92 y CI_{50} = 77,88 μg / ml respectivamente).

Palabras clave: DPPH; ABTs; SOD; Fosfomolibdeno; Helechos; Antioxidante.

Abstract

The present study was aimed to determine the free radical scavenging abilities of *Tectaria paradoxa* (TP) and *Bolbitis appendiculata* (BA) using DPPH, SOD, phosphomolybdenum and ABTs assay. The better DPPH assays IC_{50} values of TP extracts was the methanolic extract (102.25 μg/mL) and for BA extracts was acetone (121.06 μg/mL). Among the various extracts tested, methanolic extracts of BA and TP displayed highest antioxidant activity. The SOD free radical scavenging ability of TP extracts was petroleum ether IC_{50}= 123.46 μg/mL and for BA extracts was acetone IC_{50}= 108.7 μg/mL. The free radical scavenging ability of BA and TP extracts was acetone (IC_{50}= 76.92 and IC_{50}= 77.88 μg/mL respectively).

Key words: DPPH; ABTs; SOD; Phosphomolybdenum; Ferns; Antioxidant.

Introduction

Among the division of plant kingdom, lycophytes and ferns represent a group of vascular cryptogams, about 13,600 species are identified. Of which 1250 are distributed in India. Nearly 350 species are reported from Western Ghats of India. Phytochemical studies on ferns and lycophytes confirmed the occurrence of some secondary metabolites (Johnson *et al.* 2020). Phenolics, polyphenol, tannins, flavonoids, terpenoids are the most commonly reported secondary metabolites from ferns and lycophytes (Janakiraman & Johnson 2016a, Janakiraman & Johnson 2016b, Johnson *et al.* 2014, Johnson *et al.* 2020).

These metabolites have the ability to scavenge the free radicals. Due to these scavenging abilities, it generated the interest among the biologist to produce natural antioxidants. For Indian ferns and lycophytes, only very few studies are reported on the phytochemical and antioxidant activities (Janakiraman & Johnson 2015, Janakiraman & Johnson 2016a, Janakiraman & Johnson 2016b, Johnson *et al.* 2014, Johnson *et al.* 2020).

The biological activities of some Indian ferns are reported viz., anti-bacterial (Haripriya *et al.* 2010, Irudayaraj *et al.* 2010, Kwon *et al.* 2007, Vincent *et al.* 2012), anti-microbial (Jarial *et al.* 2018, Singh *et al.* 2008), anti-fungal (Raj *et al.* 2011, Raja *et al.* 2012), anti-inflammatory (Babii *et al.* 2016, Johnson *et al.* 2017, Johnson *et al.* 2020, Johnson *et al.* 2020a, Sing *et al.* 2008, Yonathan *et al.* 2006), cytotoxic (Johnson *et al.* 2014, Johnson *et al.* 2018, Radhika *et al.* 2010) anti-cancer (Bahadori *et al.* 2015, Delmas and Xiao 2012, Jarial *et al.* 2018, Nithya *et al.* 2016) anti-diabetic and anti-oxidant activity (Chen *et al.* 2015, Janakiraman & Johnson 2015, Jarial *et al.* 2018, Johnson *et al.* 2014, Sivaraman *et al.* 2013, Suzana *et al.* 2017,Wang *et al.* 2016). The free radical scavenging potentials of several ferns and lycophytes was reported by Gayathri *et al.* (2005), Lai & Lim (2011), Semwal *et al.* (2013), Lamichhane *et al.* (2014), Komala *et al.* (2015), Valizadeh *et al.* (2015), Janakiraman & Johnson (2015), Jarial *et al.* (2018), Jenat & Suresh (2018), Zhang *et al.* (2019).

In Ayurveda, *Tectaria cicutaria* (L.) Copeland decotion was used to treat gynecological disorders and inflammatory conditions (Choudhari *et al.* 2013, Upadhye *et al.* 1998). Preeti & Namdeo (2018) and Preeti & Namdeo (2018a) reported the phytoprofile, anti-microbial activity and *in vitro* anticancer activity of *T. cicutaria* rhizomes. *T. heracleifolia* total phenolic and flavonoid contents, anti-inflammatory properties and antioxidant capacity were determined by Castejón-Arroyo *et al.* (2016). The phytoconstituents of *Tectaria coadunata* (Wall. ex Hook. & Grev.) C.Chr. was determined qualitatively by Pawar *et al.* (2016). The isoperoxidase profile and interspecific variation of south Indian *Tectaria* species were reported by Johnson *et al.* (2010). Sukumaran *et al.* (2012) identified the presence of phenols, saponins, steroids, tannins, xanthoproteins, coumarins and carbohydrates in the fronds of *Tectaria zeilanica* (Houtt.) Sledge. Shrestha *et al.* (2019) and Marahatta *et al.* (2019) studied the phytoprofile, anti-bacterial and anti-oxidant potentials of *T. coadunata*. Mandadi *et al.* (2020) determined the phytoconstituents of *T. coadunata* the evaluated their anticancer, antibacterial and antioxidant properties. Canceran *et al.* (2018) revealed the phytoprofile of *Tectaria crenata* Cav. Neel *et al.* (2017) determined the anti-bacterial efficiency of *Tectaria gemmifera* (Fee.) Alston rhizomes and leaves. The qualitative and quantitative profile of alkaloids, steroids and glycosides in three *Bolbitis* species viz., *Bolbitis appendiculata* (Willd.) K.Iwats., *Bolbitis presliana* (Fee) Ching and *Bolbitis virens* (Hook. & Grev.) Schott methanolic extracts were reported by Kale (2015). Johnson (2015) reported the interspecific variation among the three *Bolbitis* species using protein profiles. But there is no report on the antioxidant properties of *T. paradoxa* and *B. appendiculata*. With this background the present study was aimed to determine the free radical scavenging abilities of *T. paradoxa* and *B. appendiculata* using DPPH, SOD, Phosphomolybdenum and ABTs assay.

Materials and methods

Collection of materials

Healthy, disease free plant samples of *T. paradoxa* and *B. appendiculata* were collected from their natural habitats Tirunelveli district, Tamil Nadu, India. To remove the soil particles and other debris, the collected plants were brought to the laboratory and washed well with running tap water for 10 min. The washed plants were blotted on the blotting paper and spread out at room temperature

under shade for a period of fifteen days. The shade dried plants were ground to fine powder using tissue blender. The powdered plants were then stored in refrigerator at 4 ºC for further use.

Preparation of extracts

30 g of dried and powder whole plant materials of *T. paradoxa* and *B. appendiculata* were extracted with 180 mL of petroleum ether, chloroform, acetone and methanol (1:6 ratio w/v) by using Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent (30 g of whole plants powdered samples were packed in the blotting paper and kept in the thimble and fixed in the soxhlet. The required amount solvents (180 ml) were kept in the distillation flask/receiving flask). The extraction was performed using the Soxhlet extractor for 8 h at a temperature not exceeding the boiling point of the solvent. Using the rotaevaporator, the excess solvents are separated from the miscella of *T. paradoxa* and *B. appendiculata* extracts and separated thick concentrated extracts. The thick concentrated extracts were kept in the vaccum chamber to condense. All extracts were frozen and freeze dried. The thick concentrated extracts paste was stored in an amber bottle and stored at 4 °C in a refrigerator for later biological activities. For quantitative analysis and biological activities, the extracts were dissolved in DMSO (w/v) (5 mg of crude petroleum ether, chloroform, acetone and methanolic extracts were dissolved in 5 mL of DMSO (w/v)).

Antioxidant activity

To know the antioxidant potentials of *T. paradoxa* and *B. appendiculata* extract, DPPH radical scavenging activity (Blois 1958), phosphomolybdenum assay (Prieto *et al.* 1999), super oxide radical scavenging activity (Robak & Gryglewski 1988) and ABTs assays (Re *et al.* 1999) with varied concentrations were carried out.

DPPH radical scavenging activity

T. paradoxa and *B. appendiculata* petroleum ether, chloroform, acetone and methanolic extracts at various concentrations (50, 100, 150, 200 µg/mL) were taken and the volume was adjusted to 100µl with methanol. About 5 ml of methanolic solution of DPPH (0.1 mM) was added to various extracts of *T. paradoxa* and *B. appendiculata* and standards (Rutin) and shaken vigorously. Negative control was prepared by adding 100 µl of methanol in 5 ml of 0.1 mM of DPPH. The tubes were allowed to stand for 20 min at 27 ºC. The absorbance of the sample was measured at 517 nm against the blank (methanol). Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of DPPH concentration. The lowest IC_{50} value of extracts indicates the highest antioxidant activity.

Phosphomolybdenum assay

The 100 µg/mL of petroleum ether, chloroform, acetone and methanolic extracts were combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM ammonium molybdenum) in a 4 ml vial. The vials were capped and incubated in a water bath at 95 ºC for 90 min. The reaction mixture were cooled at room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results are mean values expressed as g of ascorbic acid (AA) equivalents/100 g extract.

Super oxide radical scavenging activity (SOD activity)

The petroleum ether, chloroform, acetone and methanolic extracts were taken with 3 ml of SOD reaction mixture and illuminated with fluorescent lamp for 90 seconds. Super oxide radical scavenging was determined from absorption spectra at 590 nm. The SOD reaction mixture was as follows 20 mg riboflavin, 12 mM EDTA, 0.1 mg NBT prepared in sodium phosphate buffer (pH 6.0). For each concentration, Ascorbic acid blank sample was used for background subtraction. The reaction mixture without plant extracts were employed as control. The percentage inhibition activity was calculated using the formula:

$$\%\,of\ scavenging\ activity = \frac{Control\ OD - Sample\ OD}{Control\ OD}\ x\,100$$

ABTs Assay

The radical scavenging activity of petroleum ether, chloroform, acetone and methanolic extracts was analyzed by the 2, 2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) assay (ABTs). ABTs was produced by reacting 7 mM ABTs aqueous solution with 2.4 mM potassium persulfate. This mixture was kept at ambient temperature for 12-16 hours. Prior to assay, this solution was diluted in ethanol (about 1.89 v/v) and equili-

brated at 30 ºC to give an absorbance at 734 nm of 0.700 ± 0.02. After the addition of 1ml of diluted ABTs solution to 10 µl of *T. paradoxa* and *B. appendiculata* extract or Trolox standard (final concentration 0-15 µM) in ethanol, absorbance was measured at 30 ºC exactly 30 minutes after initial mixing. Appropriate solvent blank was also run. Triplicate analyses were made at each dilution of the standard and the percentage inhibition was evaluated at 734 nm. The percentage inhibition was plotted against Trolox concentration. Radical scavenging activity of the samples was expressed as IC_{50} which is the concentration of the sample required to inhibit 50% of ABTs concentration. The lowest IC_{50} value of extracts indicates the highest antioxidant activity.

Phytochemical Analysis

The total phenolic, tannin, flavonoid, terpenoids, sterols content of petroleum ether, chloroform, acetone and methanolic extracts were determined according to the method described by Siddhuraju & Becker (2003), Zhishen *et al.*(1999), Johnson *et al.* (2020b) respectively.

Quantification of total phenolic

Total phenolic content of crude extracts were determined, following the method described by Siddhuraju & Becker (2003). Briefly, 100 µg/mL of petroleum ether, chloroform, acetone and methanolic extracts were taken in the test tubes and made up to the volume of 1 ml with distilled water. Then, 0.5 ml of 1 N Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of 20 % sodium carbonate solution were added sequentially in each tube. The reaction mixture was vortexed and the test tubes were kept in the dark for 40 min, and the absorbance was recorded at 725 nm against blank. The analysis was performed in triplicates and the results were expressed as mg GAE (Gallic acid Equivalent)/g DW.

Quantification of total tannins

To quantify the total tannins, 100 mg of polyvinyl polypyrrolidine (PVPP) were put in an Eppendorf and the volume was adjusted to 1 mL with distilled water. Then, 100 µg/mL of petroleum ether, chloroform, acetone and methanolic extracts were added and incubated at 4 ºC for 4 h. PVPP precipitates the tannin content. Then, solution was centrifuged at 4000 rpm for 10 min and the supernatant was collected. The reaction mixture (supernatant) was made up to the known volume with distilled water. Then, 0.5 mL of 1 N Folin-Ciocalteu reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20 %) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min, and the absorbance was recorded at 725 nm against blank. The analysis was performed in triplicates and the results were expressed as mg GAE/g DW (Siddhuraju & Becker 2003).

Quantification of total flavonoids

The flavonoid contents of petroleum ether, chloroform, acetone and methanolic extracts were quantified, as it acts as a major antioxidant in plants, reducing oxidative stress, as per described by Zhishen *et al.*(1999). Initially, 100 µg/mL of petroleum ether, chloroform, acetone and methanolic extracts were taken in different test tubes. To each extract, 2 mL of distilled water was added. Then, 150 µL of 5 % $NaNO_2$ was added to all the test tubes followed by incubation at room temperature for 6 min. After incubation, 150 µL of $AlCl_3$ (10 %) was added to all the test tubes including the blank. All tubes were then incubated for 6 min at room temperature. Afterwards, 2 mL of 4 % NaOH was added, which was made up to 5 mL using distilled water. The reaction mixtures were vortexed well and allowed to stand for 15 min at room temperature. The appearance of pink color was recorded and measured spectrophotometrically at 510 nm. The amount of flavonoids was calculated in mg RE (Rutin Equivalent)/g DW.

Quantification of total sterols

Total sterols content was determined using the modified Liebermann-Burchard colorimetric assay (Johnson *et al.* 2020b). The Liebermann-Burchard reagent was prepared by adding 1.25 mL of concentrated H_2SO_4 to 50 mL of acetic anhydride. Liebermann-Burchard reagent (2 mL) was mixed with 100 µg/mL of petroleum ether, chloroform, acetone and methanolic extracts at different concentrations (50-200 µg/ml), stirred for 1 min and incubated at room temperature (26 ºC) for 13 min. The absorbance was measured at 650 nm, using cholesterol as standard. The total sterols content was expressed as mg/g cholesterol equivalent DW.

Quantification of total terpenoids

The terpenoids content of petroleum ether, chloroform, acetone and methanolic extracts was quantified by taking 1 mL of 2 % vanillin and adding 100 µg / mL of petroleum ether, chloro-

form, acetone and methanolic extracts were prepared in methanol, agitating in an icebath for 10 min. After agitation, all tubes were incubated at 60ºC for 20 min. in water bath. The test tubes were then cooled at 25º C for 5 min. All tubes were read at 608 nm against blank (Johnson *et al.* 2020b).

Results and discussion

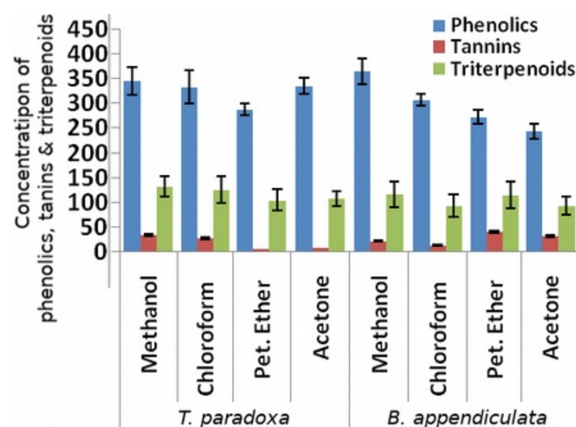
The total phenolic content of *T. paradoxa* of extracts was as follows: acetone>petroleum ether> methanol>chloroform extracs. The total phenolic content of *B. appendiculata* of extracts was as follows: acetone>chloroform>methanol>petroleum ether extract (Fig.1).

Highest amount of tannin was observed in methanolic extract of *T. paradoxa* followed by chloroform>acetone>petroleum ether extract (Fig. 1). Maximum amount of tannins was obtained in methanolic extract of *B. appendiculata* next to that petroleum ether>acetone>chloroform extract (Fig. 1).

The flavonoids and terpenoids of *T. paradoxa* were as follows chloroform>acetone> petroleum ether>methanolic extract (Figs. 1 & 2). In *B. appendiculata*, highest amount of flavonoids and terpenoids were observed in chloroform and acetone extracts respectively (Figs. 1 & 2). The petroleum ether extract of *T. paradoxa* and methanolic extract of *B. appendiculata* showed the highest amount of sterols existence.

The DPPH assays IC_{50} values of *T. paradoxa* extracts were as follows: methanolic extract (102.25 µg/mL)>acetone extract (111.86 µg/mL)>chloroform extract (136.61 µg/mL)> petroleum ether extract (163.93 µg/mL) (Fig. 4). A strong positive correlation (r=0.999) was observed between the concentration of petroleum ether extracts and scavenging activity of *T. paradoxa* followed by methanolic extracts and chloroform extracts of *T. paradoxa* also showed a positive correlation with r=0.990. Next to that acetone extracts of *T. paradoxa* also showed a positive correlation (r=0.989) against the scavenging ability.

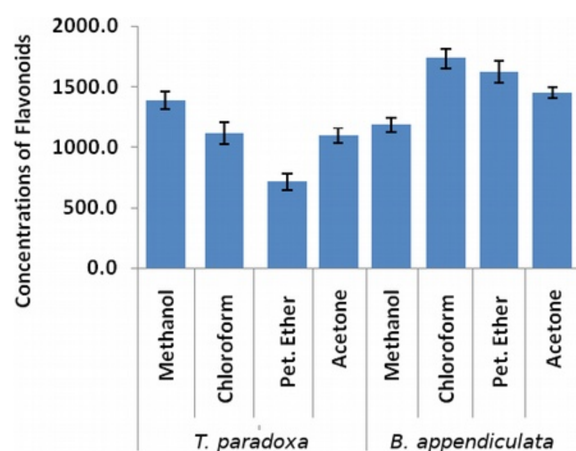
The DPPH assays IC_{50} values of *B. appendiculata* extracts were as follows: acetone (121.06 µg/mL)>methanol (123.76 µg/mL)>chloroform (139.66 µg/mL)>petroleum ether (177.94 µg/mL) (Fig. 4). A strong positive correlation (r=0.998)



Error Bar = S.D (Each and every experiments are repeated thrice Avg ± S.D)

Figura 1. Fenoles, taninos y triterpenoides totales de extractos de *T. paradoxa* y *B. appendiculata*.

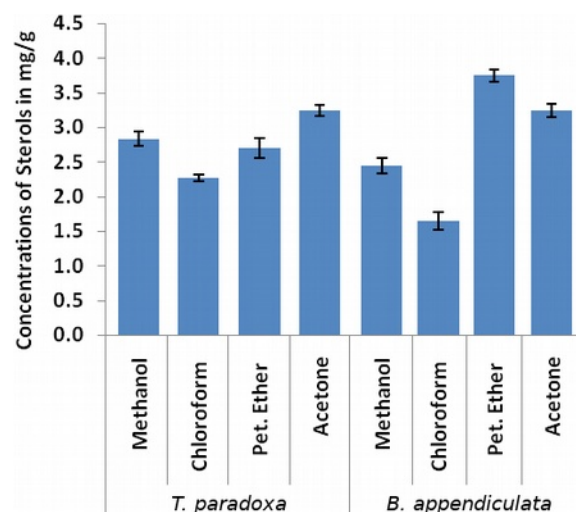
Figure 1. Total phenolics, tannins and triterpenoids of extracts of *T. paradoxa* and *B. appendiculata*.



Error Bar = S.D (Each and Every experiments are repeated thrice Avg ± S.D)

Figura 2. Flavonoides totales de extractos de *T. paradoxa* y *B. appendiculata*.

Figure 2. Total flavonoids of extracts of *T. paradoxa* and *B. appendiculata*.



Error Bar = S.D (Each and Every experiments are repeated thrice Avg ± S.D)

Figura 3. Esteroles totales de extractos de *T. paradoxa* y *B. appendiculata*.

Figure 3. Total sterols of extracts of *T. paradoxa* and *B. appendiculata*

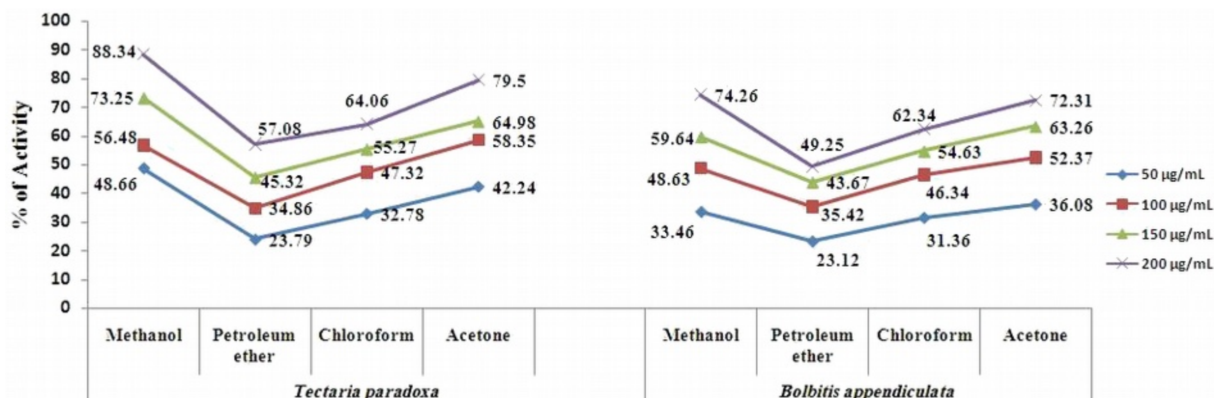


Figura 4. Actividad DPPH de *T. paradoxa* y *B. appendiculata*

Figure 4. DPPH Activity of *T. paradoxa* and *B. appendiculata*

was observed between the concentration of methanolic extracts and scavenging activity of *B. appendiculata* followed by acetone extracts of *B. appendiculata* also showed a positive correlation with r=0.991. Petroleum ether extracts of *B. appendiculata* demonstrated a positive correlation (r=0.985) between the concentration of the extracts and the scavenging ability. Similarly a positive correlation (r=0.984) was obtained between the concentrations of the chloroform extracts of *B. appendiculata* and free radical scavenging activity.

The total antioxidant ability of *T. paradoxa* and *B. appendiculata* were determined using phosphomolybdenum assay. Highest antioxidant activity was observed in *B. appendiculata*. Among the various extracts tested, methanolic extracts of *B. appendiculata* displayed highest antioxidant activity, followed by acetone extracts, petroleum ether and chloroform extracts respectively. In *T. paradoxa*, the total antioxidant activity was as follows: methanol>acetone>chloroform>petroleum ether extracts (Fig. 5).

The figure 6 explained the super oxide scavenging ability of *T. paradoxa* and *B. appendicula* extracts. A dose dependent scavenging ability was observed in *T. paradoxa* and *B. appendicula* extracts. The SOD free radical scavenging ability of *T. paradoxa* extracts were as follows acetone IC_{50}=138.89 µg/mL>chloroform IC_{50}=155.28 µg/mL>methanol IC_{50}=165. 02 µg/mL>petroleum ether IC_{50}=123.46 µg/mL. A strong positive correlation (r=0.999) was observed between the petroleum ether extracts concentrations and free radical scavenging ability of *T. paradoxa*, next to that chloroform extracts showed a correlation with r=0.988, acetone extract dispalyed r=0.984 and methanolic extracts showed the least correlation

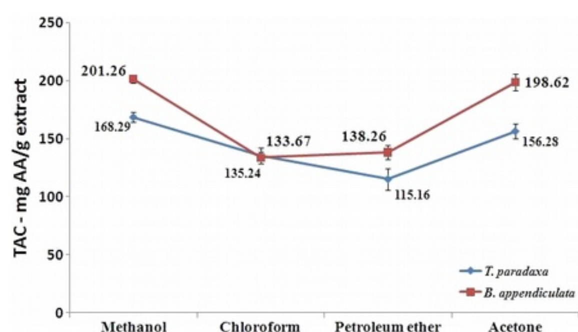


Figura 5. Actividad antioxidante total de *T. paradoxa* y *B. appendiculata*.

Figure 5. Phosphomolybdenum assay of *T. paradoxa* and *B. appendiculata*.

value r=0.937. The SOD free radical scavenging ability of *B. appendiculata* extracts were as follows acetone IC_{50}=108.7 µg/mL > chloroform IC_{50}=120.19 µg/mL>methanol IC_{50}=145.35 µg/mL>petroleum ether IC_{50}=160.26 µg/mL. A strong positive correlation (r=0.999) was observed between the concentrations of *B. appendiculata* acetone extracts and free radical scavenging ability, followed by chloroform extracts displayed r=0.996 and petroleum ether extracts showed r=0.991. The methanolic extracts of *B. appendiculata* also showed a positive correlation with r=0.978.

ABTs scavenging ability of *T. paradoxa* and *B. appendiculata* was illustatred in figure 7. A dose dependent scavenging activty was observed in *T. paradoxa* and *B. appendiculata* extracts. Among the tested two ferns, highest scavenging activity was observed in *B. appendiculata*. The free radical scavenging ability of *B. appendiculata* extracts were as follows: acetone (IC_{50}=76.92 µg/mL)>methanol (IC_{50}=77.88 µg/mL)>chloroform (IC_{50}=79.74 µg/mL)>petroleum ether (IC_{50}= 84.74 µg/mL). A strong positive correlation (r=

0.999) was observed between the concentrations of *B. appendiculata* acetone extracts and free radical scavenging ability, followed by chloroform extracts displayed r=0.991 and petroleum ether extracts showed r=0.981.

ABTs scavenging ability of *T. paradoxa* and *B. appendiculat*a was illustatred in figure 7. A dose dependent scavenging activty was observed in *T. paradoxa* and *B. appendiculata* extracts. Among the tested two ferns, highest scavenging activity was observed in *B. appendiculata*. The free radical scavenging ability of *B. appendiculata* extracts were as follows: acetone (IC_{50}=76.92 µg/mL)>methanol (IC_{50}=77.88 µg/mL)>chloroform (IC_{50}=79.74 µg/mL)>petroleum ether (IC_{50}= 84.74 µg/mL). A strong positive correlation (r= 0.999) was observed between the concentrations of *B. appendiculata* acetone extracts and free radi-

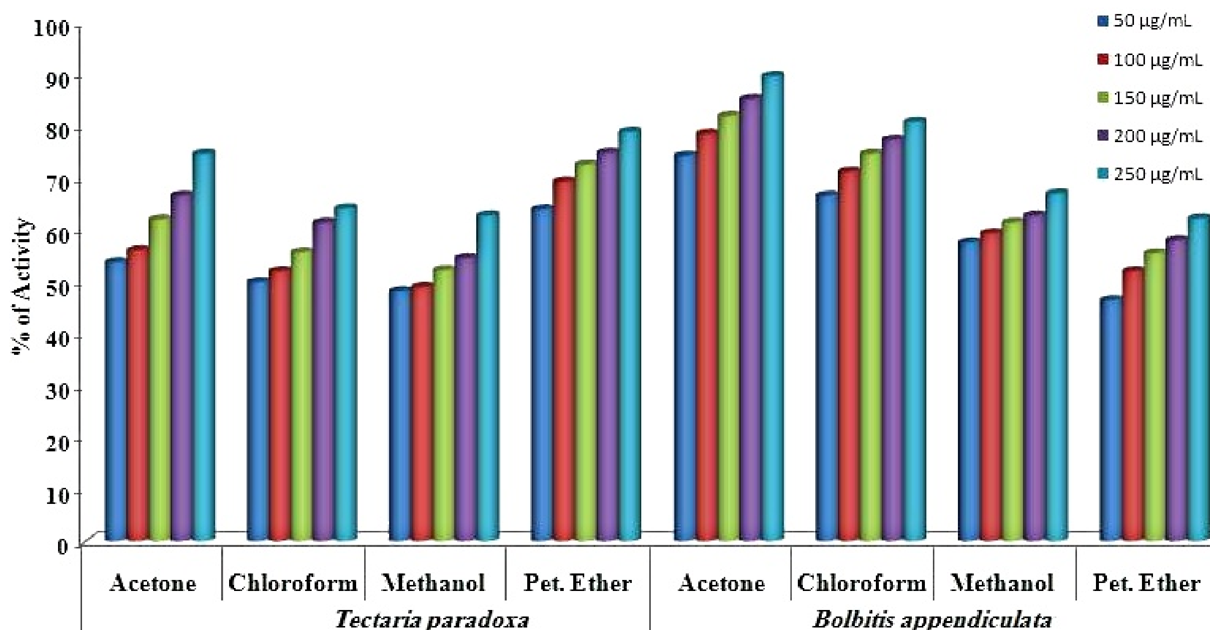


Figura 6. Actividad de eliminación de radicales superóxido de *T. paradoxa* y *B. appendiculata*.
Figure 6. SOD radical scavenging activity of *T. paradoxa* and *B. appendiculata*.

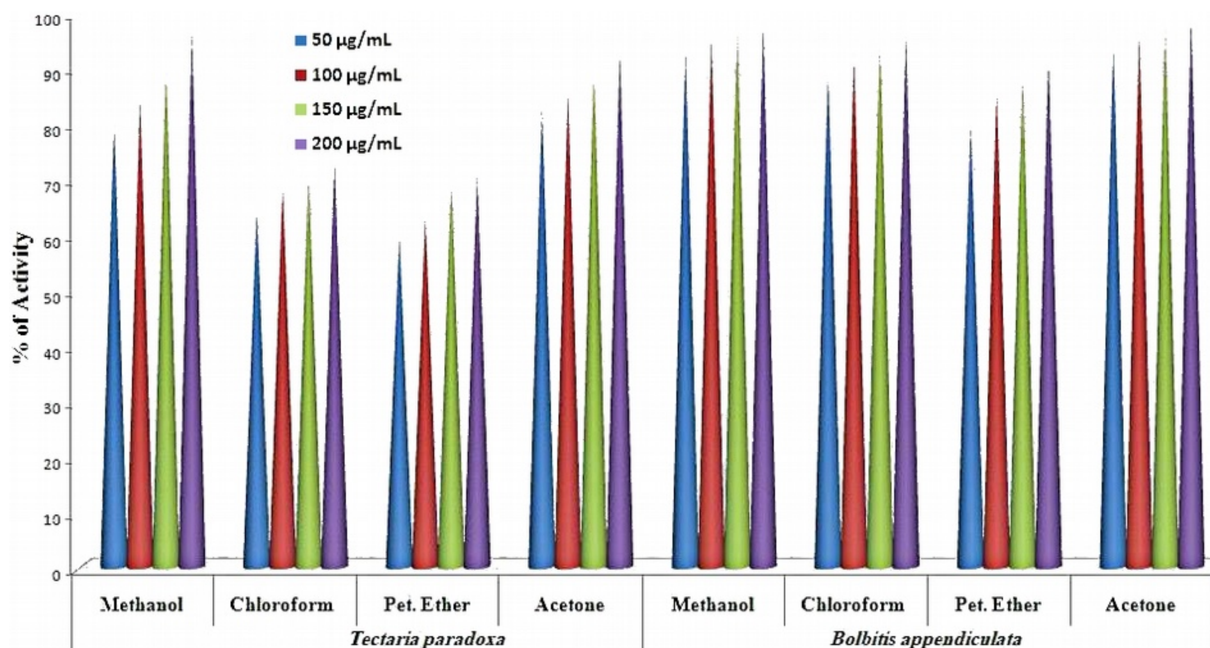


Figura 7. actividad de captación de radicales ABT de *T. paradoxa* y *B. appendiculata*
Figure 7. ABTs radical scavenging activity of *T. paradoxa* and *B. appendiculata*

cal scavenging ability, followed by chloroform extracts displayed r=0.991 and petroleum ether extracts showed r=0.981.

The methanolic extracts of *B. appendiculata* also showed a positive correlation with r=0.986. In *T. paradoxa*, the free radical scavenging ability was as follows: acetone (IC_{50}=77.88 µg/mL)> methanol (IC_{50}=83.19 µg/mL)>chloroform (IC_{50}= 106.61 µg/mL)>petroleum ether (IC_{50}=111.11µg/mL). A strong positive correlation (r=0.997) was observed between the concentrations of *T. paradoxa* acetone extracts and free radical scavenging ability, followed by methanolic extracts displayed r=0.994 and chloroform extracts showed r=0.982. The petroleum ether extracts of *T. paradoxa* also showed a positive correlation with r=0.976. The correlation is significant at the 0.05 level (2-tailed).

Due to the advancement in the instrumentation, biologists are given much focus on botanical and herbal plant resources, to find a wide array of fraction and isolated natural compounds. However, only few screening approaches have been attempted for Indian pteridophytes. In the present study, an attempt is made to reveal the secondary metabolites profile of *T. paradoxa* and *B. appendiculata*.

The phytochemical study confirmed the existence of flavonoids, phenolics, tannins, terpenoids and sterols with varied quantity in *T. paradoxa* and *B. appendiculata* (Figs. 1-3). Adil *et al.* (2010), Bagiu and Butnariu (2012), Jarial *et al.* (2018) revealed the anti-oxidative, anti-fungal, anti-inflammatory and diuretic of *Cheilanthes tenuifloia* (Burm.fil.) Sw. flavonoids and correlated the relationshipship between the concentrations of flavonoids and antioxidant properties. In the present study also the flavonoids are rich in *B. appendiculata* and *T. paradoxa* chloroform and acetone extracts and they showed good free radical scavenging ability (Figs. 4-7). The results of the present study directly coincided with Adil *et al.* (2010), Bagiu & Butnariu (2012), Jarial *et al.* (2018) observations.

The quercetin and rutin was displayed the DPPH radical scavenging activity of 86.1% and 73.2% respectively (Jarial *et al.* 2018). In the present study, the crude methanolic extracts of *B. appendiculata* and *T. paradoxa* showed 74.26 % and 88.34% DPPH radical scavenging activity respectively (Fig. 4). Significant *in vitro* anti-oxidant activity was observed in *T. paradoxa* and *B. appendicualta* methanolic extracts. The crude methanolic extracts of *T. paradoxa* and *B. appendicualta* may have shown more activity than rutin and quercetin. Antioxidant activity can be attributed by the presence of high phenolics' content (Suzana *et al.* 2017).

They observed that gametophytes of *Ceterach officinarum* Willd. (as *Asplenium ceterach*) displayed lower TPC (Total Phenolic Content) (~51 mg GAE/g FW) and thus lower antioxidant activity in DPPH and ABTs assays than sporophytes of C. *officinarum* (~232 mg GAE/g FW). In the present study also phenol rich acetone extracts of *T. paradoxa* and *B. appendicualta* (Fig. 1) showed high frequency of free radical scavenging ability (ABTs assay-Fig. 7; SOD assay-Fig. 6 and DPPH assay-Fig. 4). In phosphomolybdenum assay, also next to methanolic extracts, acetone extracts of *T. paradoxa* and *B. appendicualta* showed high rate of antioxidant activity (Fig. 5). The results of the present study also directly coincided with Suzana *et al.* (2017) observations. The available scientific evidences proved that plant phenolics efficient radical scavengers and metal chelators (Hutadilok-Towatana *et al.* 2006, Juntachote and Berghofer 2005, Lai and Lim 2011, Lim *et al.* 2007, Suzana *et al.* 2017).

The results of the present study is supplemented the previous observation and confirmed the scavenging potential of phenolics. Analysis on the correlations between concentrations of the petroleum ether, acetone, chlorofrom and methanolic extracts (phenols/tannins/flavonoids) and the free radical scavening abilities/antioxidant properties measured good correlations between petroleum ether, acetone, chloroform and methanolic extracts of *T. paradoxa* and *B. appendiculata* and DPPH, SOD and ABTs assays. The observed results suggest that phenolic and flavonoids compounds are powerful scavenger of free radicals as well as reducing agents. Similar trend was observed in the previous studies also (Johnson *et al.* 2014, Kumar *et al.* 2008, Miliauskas *et al.* 2004, Suzana *et al.* 2017).

Conclusion

The acetone and methanolic extracts of *T. paradoxa* and *B. appendiculata* demonstrated very high total phenolic content and high radical scavenging capacity; the observations clearly concluded that they are potent primary antioxidants.

The results suggest that acetone and methanolic extracts of *T. paradaxa* and *B. appendiculata* could potentially be employed in traditional medicine as they are rich in compounds with antioxidant properties and lead to use as potential natural antioxidants in the pharmaceutical and nutraceutical industries.

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