VOLUME 9 (SPECIAL ISSUE 3) 2023

ISSN 2454 - 3055

Manuscripts under the Special Issue are published under the theme "COMPLEMENTARY AND ALTERNATIVE THERAPEUTIC TECHNIQUES"

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INTERNATIONAL JOURNAL OF ZOOLOGICAL INVESTIGATIONS

Forum for Biological and Environmental Sciences
Published by Saran Publications, India

International Journal of Zoological Investigations Vol. 9 (Special Issue 3), 67-75 (2023)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

Editor-in-Chief: Prof. Ajai Kumar Srivastav

Published by: Saran Publications, Gorakhpur, India

ISSN: 2454-3055

Evaluation of Antioxidant Activity of Different Extracts of *Scoparia dulcis* Whole Plant

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Received: 12th November, 2023; **Accepted:** 15th December, 2023; **Published online:** 27th December, 2023

https://doi.org/10.33745/ijzi.2023.v09ispl3.010

Abstract: Plant derived antioxidant could be useful as food additives to prevent food deterioration and also to impart human health and prevent oxidative stress associated diseases. In this study, the results of the evaluation of phytochemical screening of aqueous, ethanol methanolic and hydroalcohol extracts of *Scoparia dulcis* revealed the presence of tannin, saponin, flavonoids, phytosterols, alkaloids and phenol but diterpenes, glycoside were present in methanolic and hydro-alcohol extracts of *Scoparia dulcis.* The potential therapeutic benefit of different extracts (Hydro-alcohol, methanol, ethanol, and aqueous) of *Scoparia dulcis* whole plant was prepared for *in vitro* antioxidant models as DPPH radical scavenging, nitric oxide, ferric reducing power activity and Phosphorus molybdenum assay. The IC_{50} values of DPPH are- hydro-alcohol (146.92 µg/ml) > methanol (166.23 µg/ml) >ethanol (182.20 µg/ml) >aqueous (189.17 µg/ml), respectively and ascorbic acid (134.32 µg/ml). The IC_{50} values of nitric oxide scavenging activity are- hydro-alcohol (131.62 µg/ml) > methanol (154.78 µg/ml) > ethanol(166.40 µg/ml) > aqueous(178.64 µg/ml), respectively and ascorbic acid (124.94 µg/ml). The IC_{50} values of FRAP are- hydro-alcohol (138.37 µg/ml), methanol (150.83 µg/ml) > ethanol (163.70 µg/ml) > aqueous (181.59 µg/ml), respectively and ascorbic acid (123.74 µg/ml). The antioxidant activity of *Scoparia dulcis* whole plant has maximum activity in hydro-alcoholic extract followed by methanol, ethanol and aqueous extracts. *Scoparia dulcis* whole plant could potentially serve as prospective material for further development of novel and safer plant-based agents with antioxidant effect. The phytochemical components of medicinal plants are responsible for their therapeutic properties, particularly those with antioxidant properties.

Keywords: *Scoparia dulcis*, Phytochemicals, Antioxidant properties, DPPH, Nitric oxide, Ferric reducing power activity

Citation: Manoranjitha M. and Malarvizhi A.: Evaluation of antioxidant activity of different extracts of *Scoparia dulcis* whole plant. Intern. J. Zool. Invest. 9(Special Issue 3): 67-75, 2023.

https://doi.org/10.33745/ijzi.2023.v09ispl3.010

Introduction

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants. Antioxidants are

vital substances, which possess the ability to protect the body from damages caused by free radical-induced oxidative stress (Kanhiya Mahour, 2023). The antioxidant present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of substrate (Yang *et al*., 2017). The term 'oxidizable substrate'includes almost everything found in the living cells including proteins, lipids, DNA and carbohydrates. Biological antioxidants have been defined as compounds that protect biological systems against the potentially harmful effects of processes or reaction that can cause excessive oxidation (Kasote *et al*., 2015). Our body is rich in endogenous antioxidants, the substances that have the ability to stop free radicals formation or to limit the damage they cause (Otitolaiye *et al*., 2023). Free radicals are reactive molecules involved in many physiological processes and have been associated with many diseases, such as cancer, arthritis and liver injury. As a result, there is need to explore substances with free radical scavenging and/ or antioxidant activity (Arogba *et al*., 2012).

Antioxidant compounds in food play an important role as a health protecting factor. Primary sources of naturally occurring antioxidants are whole grains, fruits and vegetables. Highly reactive free radicals and oxygen species can initiate various diseases (Adebiyi *et al*., 2017). Free radicals arising from metabolism or environmental sources interact continuously in biological systems and their uncontrolled generation correlates directly with molecular level of many diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis (Nur Alam *et al.*, 2013; Mwamatope *et al*., 2020;). Free radicals would damage nearby structures including DNA, proteins or lipids. Radical scavenging antioxidants are particularly important in antioxidative defence in protecting cells from the injury of free-radical. Plants are the good source of biologically active compounds known as phytochemicals. The phytochemicals have been found to act as antioxidants by scavenging free radicals and may have therapeutic potential for free radical associated disorders (Anokwuru *et al*., 2011; Otitolaiye *et al*., 2019). Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.). The present study was aimed to carry out the *in vitro* antioxidant activity by DPPH (1, 1-diphenyl-2-picryl-hydrazyl) radical, Nitric oxide and ferric reducing power activity models using the different extracts of *Scoparia dulcis* whole plant.

Materials and Methods

Collection of plant materials:

The whole plant of *Scoparia dulcis* were collected from Thiruppanandal, Thanjavur (District), Tamil Nadu, India from a single herb. The plant of *Scoparia dulcis* was identified and authenticated by Dr. K. Sankarganesh. Department of Botany. D. G. Government Arts College for women, Mayiladuthurai. Tamil Nadu. India.

Preparation of extracts:

The collected *Scoparia dulcis* plants were washed several times with distilled water to remove the traces of impurities from the plant. The whole plant was dried at room temperature and coarsely powdered. The powder was extracted with ethanol, methanol, hydro-alcohol and aqueous using Soxhlet extraction. A semi solid extracts were obtained after complete elimination of solvent under reduced pressure. The *Scoparia dulcis* extract was stored in refrigerator until used for *in vitro* antioxidant activity.

Quantitative analysis:

The total phenolic content for all the solvents were determined using the Folin–Ciocalteu method. The total flavonoid content for all the solvents were determined using spectrophoto-metric method. Total saponins contents were

Table 1: Phytochemical analysis of different extracts of *Scoparia dulcis* L.

S. No.	Phytochemicals	Aqueous extract	Ethanol extract	Methanol extract	Hytro-alcohol extract
1	Tannin	+	+	++	++
2	Saponin	+	+	++	++
3	Flavonoids	+	++	++	++
4	Phytosterols	+	+	++	++
5	Diterpenes	-	-	+	++
6	Alkaloids	+	+	+	+
7	Phenol	++	++	++	++
8	Glycoside	-	-	+	++

(+) Present, (++) High concentrations, (-) Absent

Table 2: Quantitaive analysis of phytochemicals in *Scoparia dulcis* L.

Extracts	Total phenol (mg GAE/g dry weight)	Total flavonoids (mg QE/g dry weight)	Saponin (Milligrams of saponin /g dry weight)	Alkaloid (Milligrams of atropine/g dry weight)
Ethanol	151.72±10.57	110.81±7.70	93.67±6.51	49.05±3.43
Methanol	163.14±11.41	119.75±8.33	98.47±6.86	52.48±3.64
Aqueous	139.95±9.73	104.47±7.28	82.49±5.74	45.16±3.15
Hydro-alcohol	186.24±13.02	128.33±8.96	108.38±7.56	58.65±4.06

Values are expressed as mean ± Standard deviation (n=3)

estimated by colorimetric methods (Hiai *et al*., 1976). The alkaloid estimated by the method of Fazel Shamsa *et al.* (2008) and Mallikarjuna Rao *et al.* (2012).

In vitro antioxidant activity:

The effect of *Scoparia dulcis* extract on DPPH radical was estimated using the method of Liyana-Pathiranan *et al*. (2005). Determination of nitric oxide radical scavenging activity of *Scoparia dulcis* was prepared by method of Bajpai *et al*. (2013). The Benzie and Strain, (1996) technique was used to assess the FRAP activity of *Scoparia dulcis*. Phosphorus molybdenum assay was determined by the method of Bhatti *et al*. (2015).

Statistical analysis:

Tests were carried out in triplicate for 3 separate experiments. The amount of sample needed to inhibit free radicals concentration by 50%, IC_{50}, was graphically determined by a linear regression method using MS- Windows based Graph Pad Instat (version 3) software. Results are expressed as graphically/mean ± standard deviation.

Results and Discussion

Phytochemical analysis:

The results of the evaluation of phytochemical screening of aqueous, ethanol, methanolic and hydro-alcohol extracts of *Scoparia dulcis* revealed the presence of tannin, saponin, flavonoids, phytosterols, alkaloids and phenol but diterpenes, glycoside were present in methanolic and hydroalcohol extracts of *Scoparia dulcis.* Diterpenes and glycosides were absent only in the aqueous and ethanol extracts. The intensity of the colour observed from the study was used to indicate whether the phytochemicals were present (+), absent (-) or high concentration (++). Among the various extracts, the hydro-alcoholic extract of *Scoparia dulcis* contains a higher concentration of phytochemicals compared to aqueous, ethanol and methanolic extracts of *Scoparia dulcis* (Table 1).

Quantitative analysis:

Table 2 represent the quantitative analysis of phytochemicals as total phenol, total flavonoids,

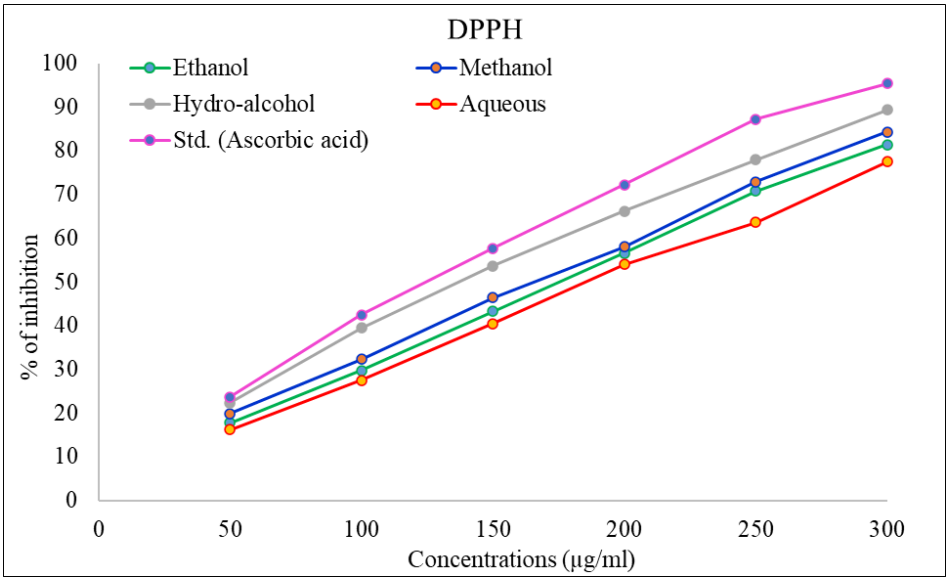

Fig. 1: DPPH radical scavenging activity of different extracts of *Scoparia dulcis.*

Table 3: DPPH radical scavenging activity of different extracts of *Scoparia dulcis*

Samples	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	300 (µg/ml)	IC_{50} (µg/ml)
Ethanol extract	17.74±2.40	29.73±2.08	43.23±2.08	56.52±2.75	70.76±2.43	81.34±4.04	182.20
Methanol extract	19.91±1.46	32.34±3.65	46.40±0.97	58.05±1.82	72.88±2.55	84.33±2.55	166.23
Aqueous extract	16.20±2.74	27.44±3.41	40.43±2.09	54.04±2.92	63.60±4.04	77.45±3.24	189.17
Hydro-alcohol extract	22.29±0.46	39.40±4.22	53.63±2.68	66.14±2.88	77.97±3.20	89.30±2.05	146.92
Std. (Ascorbic acid)	23.68±0.93	42.47±2.63	57.72±3.47	72.20±1.69	87.17±2.35	95.38±3.20	134.32

Values are expressed as mean ± Standard deviation (n=3); IC: Inhibition concentration

alkaloids and saponin in *Scoparia dulcis*. Significant amount of phenol (Aqueous: 139.95, ethanol: 151.72, methanol: 163.14 and hydro-alcohol: 186.24 mg GAE/g dry weight) and flavonoids (Aqueous: 104.47, ethanol: 110.81, methanol: 119.75 and hydro-alcohol: 128.33 mg QE/g dry weight) were present. Significant amount of saponin (Aqueous: 86.49, ethanol: 93.67, methanol: 98.47 and hydro-alcohol: 104.38 mg saponin/g dry weight) and alkaloids (Aqueous: 44.16, ethanol: 49.05, methanol: 52.48 and hydro-alcohol: 58.65 mg atropine/g dry weight) were present (Table 2; Fig. 1). Among the various extracts, the hydro-alcoholic extract of *Scoparia dulcis* contains a higher amount of phenol, flavonoids, saponin and alkaloids were compared to aqueous, ethanol and methanol extracts. On the basis of qualitative and quantitative analysis, the rich content of phytochemicals present in hydro-alcohol extract was observed in this study.

In vitro antioxidant activity:

DPPH radical scavenging activity:

1,1- Diphenyl-2-picrylhydrazyl (DPPH) radical is a commonly used method to assess the free radical scavenging ability of various samples. The DPPH radical scavenging activity results are shown in Figure 1 and Table 3 as comparable with known antioxidant ascorbic acid. The ethanol, methanol, aqueous and hydro-alcohol extracts of *Scoparia dulcis* also demonstrated

Table 4: Nitric oxide scavenging activity of different extracts of *Scoparia dulcis*

Samples	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	300 (µg/ml)	IC_{50} (µg/ml)
Ethanol extract	19.22±2.15	30.52±2.42	49.25±3.32	59.25±4.32	72.47±2.12	82.72±2.39	166.40
Methanol extract	20.73±1.24	35.37±2.29	53.17±3.60	61.32±4.16	74.78±4.31	85.93±2.85	154.78
Aqueous extract	17.11±2.97	28.65±1.66	46.02±1.58	55.19±2.58	69.19±2.00	78.30±1.12	178.64
Hydro-alcohol extract	24.16±1.88	41.48±3.23	59.48±0.99	72.45±2.01	80.72±2.35	90.32±1.30	131.62
Std. (Ascorbic acid)	24.21±2.60	44.51±3.05	62.46±2.14	74.13±2.86	82.11±2.10	92.70±2.89	124.94

Values are expressed as mean ± Standard deviation (n=3); IC: Inhibition concentration

remarkable *in vitro* DPPH radical scavenging activity in a dose-dependent trend. The higher scavenging activity was recorded on the hydro-alcoholic extract at 300 µg/ml for *Scoparia dulcis* (89.30±2.05%), which is comparable to the positive control ascorbic acid (95.38±3.20%). The higher the concentration of the extracts, the more powerful the ability of the extract to give out a hydrogen atom to the radical. The following order of extracts showed the radical scavenging activity of DPPH with IC_{50} values are hydro-alcohol (146.92 µg/ml) > methanol (166.23 µg/ml)> ethanol (182.20 µg/ml) >aqueous (189.17 µg/ml) respectively and ascorbic acid (134.32 µg/ml). The lowest IC_{50} value has greatest antioxidant activity. Among the various extracts, hydro-alcoholic extract possess potential activity than other extracts.

DPPH is a nitrogen centred radical and the change of colour from violet to yellow occur upon reduction by the process of hydrogen or electron donation. Substances are ability to perform this reaction that can be measured as antioxidants and thus radical scavengers. It was observed that the free radical scavenging activities of *Scoparia dulcis* whole plant extract increased with increasing concentration. The antioxidant substance present in the extract counters with DPPH free radical solution and converts them into reduced form either by transferring electron or donating hydrogen atom followed by proton (Nuutila *et al.,* 2003).

Nitric oxide scavenging activity:

Nitric oxide is very important in both cell signaling and oxidative/nitrosative stress and can create the extremely reactive peroxynitrite anion ($ONOO^-$) when reacted with superoxide radical. Furthermore, nitric oxide generation has been linked to an increase in the production of proinflammatory mediators including cytokines and reactive oxygen species, which can worsen inflammatory diseases like ulcerative colitis, diabetes, multiple sclerosis, and arthritis (Otitolaiye *et al*., 2023).

The nitric oxide scavenging activity results are shown in Figure 2 and Table 4 as comparable with known antioxidant ascorbic acid. The ethanol, methanol, aqueous and hydro-alcohol extracts of *Scoparia dulcis* also demonstrated remarkable *in vitro* nitric oxide scavenging activity in a dose-dependent trend. At all the studied concentrations, the hydro-alcohol extract of *Scoparia dulcis* produced significantly higher nitric oxide scavenging activity than those recorded for the methanol, ethanol and aqueous extracts of *Scoparia dulcis.* The maximum activity was observed in hydro-alcoholic extract at 300 µg/ml for *Scoparia dulcis* (90.32±1.30%), which is comparable to the positive control ascorbic acid (92.70±2.89%). The extracts showed potent scavenging activity of nitric oxide with IC_{50} values in the order of hydro-alcohol (131.62 µg/ml) > methanol (154.78 µg/ml) > ethanol (166.40 µg/ml) > aqueous (178.64 µg/ml), respectively and

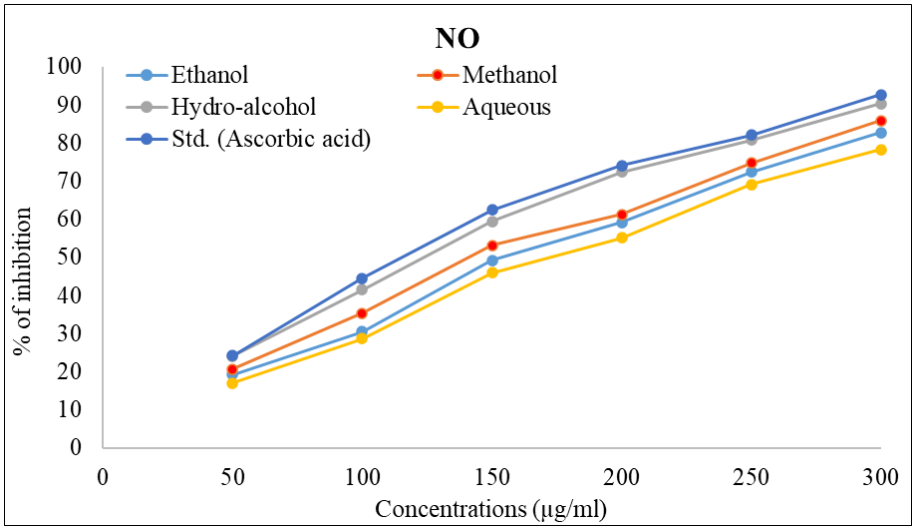

Fig. 2: Nitric oxide scavenging activity of different extracts of *Scoparia dulcis.*

Table 5: Ferric reducing antioxidant power assay

Samples	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	300 (µg/ml)	IC_{50} (µg/ml)
Ethanol extract	20.99±1.48	34.06±3.67	49.08±1.86	58.98±3.68	72.53±2.23	80.71±2.28	163.70
Methanol extract	22.52±3.17	38.13±2.72	54.65±3.16	62.65±1.92	73.04±2.54	84.06±4.18	150.83
Aqueous extract	17.67±3.09	31.77±1.44	45.53±2.04	54.13±3.23	66.01±5.91	75.81±2.88	181.59
Hydro-alcohol extract	23.68±1.51	41.54±2.98	57.74±1.95	66.86±1.35	77.08±3.60	87.99±1.62	138.37
Std. (Ascorbic acid)	26.25±1.20	43.73±2.46	60.74±2.45	73.30±2.74	85.82±0.46	94.64±4.09	123.74

Values are expressed as mean ± Standard deviation (n=3); IC: Inhibition concentration

ascorbic acid (124.94 µg/ml). The lowest IC_{50} value has greatest antioxidant activity. Among the various extracts, hydro-alcoholic extract possess potential activity than other extracts.

The nitric oxide quenching potential of the aqueous and ethanol extracts of *Scoparia dulcis* was assessed by using Griess reagent. Nitric oxide is produced by sodium nitroprusside in an aqueous solution, and it can react with oxygen to produce nitrite ions (radicals). This in turn can be quenched by the antioxidants in the extracts which donate protons to the nitrite ions. As such, the stronger potential of *Scoparia dulcis* extracts to inhibit the production of nitric oxide radicals could have positive implications for the ability of this plant to ameliorate oxidative stress mediated diseases.

Ferric reducing antioxidant power (FRAP) assay:

The FRAP assay measures the reducing potential of an antioxidant reacting with a ferric tripyridyltriazine (Fe^{3+}-TPTZ) complex and produce a coloured ferrous tripyridyltriazine (Fe^{2+}-TPTZ). Generally, the reducing properties are linked with the presence of compounds which exert their action by breaking free radical chain by donating a hydrogen atom (Duh *et al.*, 1999). The FRAP results are shown in Figure 3 and Table 5 as comparable with known antioxidant ascorbic acid. The ethanol, methanol, aqueous and hydro-alcohol extracts of *Scoparia dulcis* also demonstrated

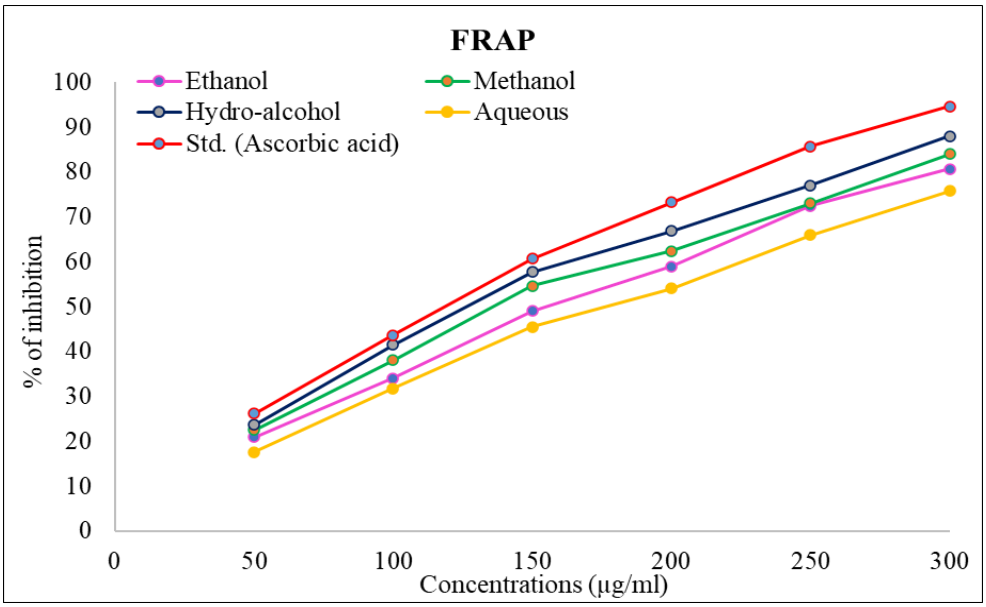

Fig. 3: Ferric reducing antioxidant power assay of different extracts of *Scoparia dulcis.*

Table 6: Phosphomolybdenum assay of different extracts of *Scoparia dulcis*

Samples	50 (µg/ml)	100 (µg/ml)	150 (µg/ml)	200 (µg/ml)	250 (µg/ml)	300 (µg/ml)	IC_{50} (µg/ml)
Ethanol extract	18.71±2.24	31.07±1.40	43.44±2.02	60.59±2.99	71.02±1.85	82.32±2.86	170.43
Methanol extract	20.63±1.98	34.87±2.02	48.26±3.82	65.46±2.30	75.87±3.42	86.07±3.31	155.53
Aqueous extract	15.49±1.95	28.82±1.68	38.41±2.76	57.99±1.45	64.71±2.41	77.29±3.68	186.55
Hydro-alcohol extract	22.95±2.54	36.65±2.59	52.26±3.19	67.20±2.54	79.89±4.60	89.33±1.97	142.86
Std. (Ascorbic acid)	25.27±1.20	40.58±2.46	56.99±2.45	72.57±2.74	83.13±0.46	92.43±4.09	131.77

Values are expressed as mean ± Standard deviation (n=3); IC: Inhibition concentration

remarkable *in vitro* FRAP in a dose-dependent trend. The hydro-alcohol extract of *Scoparia dulcis* produced significantly higher FRAP than those recorded for the methanol, ethanol and aqueous extracts of *Scoparia dulcis*. The extract showed potent scavenging activity of FRAP with IC_{50} values in the order of hydro-alcohol (138.37µg/ml), methanol (150.83 µg/ml) > ethanol (163.70 µg/ml) > aqueous (181.59 µg/ml), respectively and ascorbic acid (123.74 µg/ml). The lowest IC_{50} value has greatest antioxidant activity. Among the various extracts, hydro-alcoholic extract possess potential activity than other extracts.

In the present study, the absorbance of *Scoparia dulcis* clearly increased, due to the formation of the Fe^{2+}-TPTZ complex with increasing concentration as seen also in the reference antioxidant as ascorbic acid. Hence, the plant is able to donate electrons to free radicals stable in the actual biological and food systems (Benzie and Strain, 1996). The extracts of *Scoparia dulcis* showed a considerable antioxidant effect in all the extractions.

Phosphomolybdenum assay:

The phosphomolybdenum assay results are shown in Figure 4 and Table 6 as comparable with known

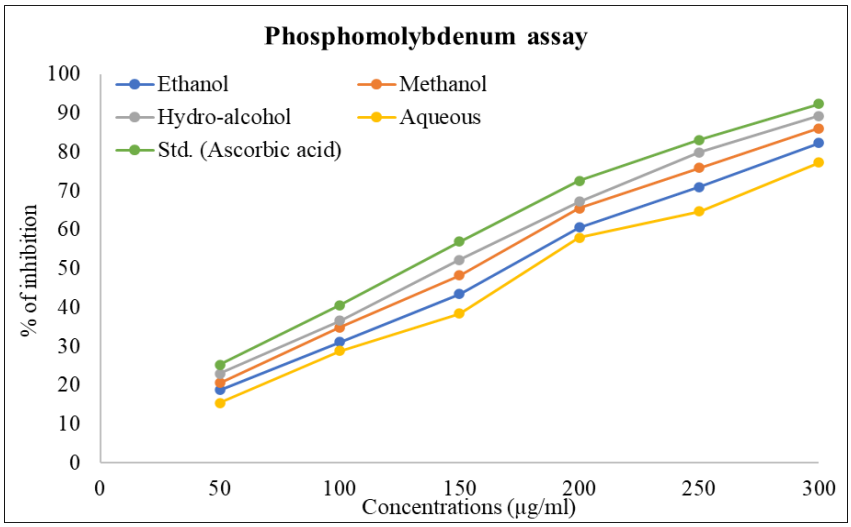

Fig. 4: Phosphomolybdenum assay of different extracts of *Scoparia dulcis.*

antioxidant ascorbic acid. The ethanol, methanol, aqueous and hydro-alcohol extracts of *Scoparia dulcis* also demonstrated remarkable *in vitro* phosphomolybdenum in a dose-dependent trend. At all the studied concentrations, the hydro-alcohol extract of *Scoparia dulcis* produced significantly higher phosphomolybdenum than those recorded for the methanol, ethanol and aqueous extracts of *Scoparia dulcis*. The extract showed potent scavenging activity of phosphomolybdenum with IC_{50} values in the order of hydro-alcohol (142.86 µg/ml), methanol (155.53 µg/ml) > ethanol (170.43 µg/ml) > aqueous (186.55 µg/ml), respectively and ascorbic acid (131.77 µg/ml). The lowest IC_{50} value has greatest antioxidant activity. Among the various extracts, hydro-alcoholic extract possess potential activity than other extracts.

The findings of this investigation also demonstrated that the inhibition efficiency of DPPH, nitric oxide, ferric reducing power activity and Phosphomolybdenum assay increased as the extracts' amount increased. This supports the finding by Otitolaiye *et al*. (2023) and Hussen Endalew (2023) as they observed the maximum radical scavenging activity in highest concentration of extracts.

Conclusion

It can be concluded that all the solvent extracts of *Scoparia dulcis* possess some degree of antioxidant potential. The maximum antioxidant activity was noticed in *Scoparia dulcis* hydro-alcohol extract followed by methanol, ethanol and aqueous extract using *in vitro* models as DPPH radical scavenging, nitric oxide, ferric reducing power activity and phosphomolybdenum assay. The antioxidant activity of the extracts may be due to the presence of phytochemicals. Therefore, *Scoparia dulcis* whole plant extracts have considerable antioxidant properties and the consumption of this under-exploited plant may play a role in preventing human diseases in which free radicals are involved, such as cancer, cardiovascular disease, and premature aging.

Acknowledgements

The authors are thankful to The Principal, D. G. Govt. Arts College, Mayiladuthurai, India for providing all facilities to perform this work.

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