Antioxidant Compound Quercetin-3-O-α-L-rhamnoside(1→6)-β-D-glucose (Rutin) isolated from ethyl acetate leaf extracts of *Memecylon edule* Roxb (Melastamataceae)

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ABSTRACT

Introduction: The present study was aimed to isolate antioxidant principle from leaf ethyl acetate extract of *Memecylon edule* for the first time. *M. edule* belongs to melastomataceae family; possess various biological properties and folklore uses. **Methods:** Isolation of antioxidant compound was carried out by DPPH radicals scavenging activity guided method and employing chromatography techniques (TLC and column chromatography). The structure of isolated bioactive compound (Rutin) was confirmed by using various spectral data like UV, FT-IR, LC-MS, CHNS analysis, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, HMBC and HSQC**.** *In vitro* antioxidant activity of isolated compound was evaluated by using DPPH radical, nitric oxide radical, superoxide radical, hydroxyl radical scavenging assays and ferric reducing antioxidant power assay (FRAP) with reference to ascorbic acid and butylated hydroxyanisole (BHA). One way analysis of variance (ANOVA) and Tukey's multiple comparison tests were performed. **Results:** DPPH activity guided isolation resulted isolation of Rutin. Isolated rutin exhibited remarkable scavenging capacity on all tested radicals in a concentration dependent manner. The results also showed that Rutin harbor strong reductive capacity on hydroxyl radicals with lowest IC_{50} value (17.06 µg/ml) and strong ferric ion (Fe^{3+}) chelation (EC_{50} value 17.29 µg/ml) potential. **Conclusion:** The results of the present investigation concluded that the leaf ethyl acetate extract of *M. edule* is a good source of Rutin which harbor significant antioxidant potential.

Key words: Antioxidant activity, HMBC, HSQC, *Memecylon edule,* Rutin.

INTRODUCTION

Free radicals such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) are known to causes damage to the cellular biomolecules (DNA, proteins, lipids, amines and carbohydrates), resulting in ageing and other degenerative diseases such as cancers, diabetes, Alzheimer's disease and Parkinson's disease etc.[1] Antioxidants have been reported to prevent oxidative damage caused by free radical, it can interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers.[2] Imbalance of free radicals and antioxidant rustled oxidative stress. Hence, certain amounts of exogenous anti-oxidants are constantly required to maintain an adequate level of anti-oxidants in order to balance the ROS in human body. In this concern, synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT) are widely used but they are potential to causes health risk like carcinogenicity and toxicity. Hence, the attentions of investigators are turning to find out the natural antioxidants which are much safer

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and cheap.[3] Epidemiological studies have been reported that phytonutrients are beneficial in protecting human body against oxidative damage caused by free radicals. Therefore, increases the interest in isolation and characterization of plant-derived antioxidants.[4] *Memecylon edule* Roxb. (Melastamataceae family) is an evergreen tree found in Eastern and Western Ghats of India. The plant has dyeing property.[5] The leaves of this plant used for the treatment of ophthalmic, leucorrhoea, spasmolysis, gonorrhea and hypoglycaemia[6] and possess anti-inflammatory, analgesic, wound healing and free radical scavenging properties.[7,8] The roots of this plant used as a remedy for excessive menstrual discharge.[9] Root and leaf decoction used for food poisoning and treat urticaria antipruritic in Thai medicine.[10] Roots and heart wood extract has been used to get relief from fever symptoms of several diseases such as the common cold, measles, chicken pox.[11] The strong antibacterial activities were found in leaf extracts of *M. edule*.[5,12,13] Secondary metabolites of *M. edule* showed the presence of flavonoids and triterpenoids in crude ethyl acetate extracts of leaves. The chemical constituents (Epigallocatechin gallate, marketing and ellagic acid glycosides) of *M. edule* were isolated and tested for anti-inflammatory activity.[8] Phytochemical analysis results show the presence of 13 fatty acids, 12 methyl tetradeconate, glucose, amino acids, carotenoids, glycosides and saponins in the genus *Memecylon*.[14] Therefore, in the light of knowledge that *M. edule* having wide folklore uses we are aiming to isolate antioxidant principle from leaf extracts of *M. edule* by DPPH radical scavenging activity guided method.

MATERIALS AND METHODS

General

The purity of isolated compound was confirmed by analytical LC (Thermo/Finnegan Surveyor System) which eluted with methanol/water and LC column outlet was coupled to a Thermo fleet (LCQ-Fleet) Ion Trap mass spectrometer equipped with an ESI ion source. Data acquisition and mass spectrometric evaluation were carried out in Qual Browser; Thermo Electron, San Jose, CA. Melting point was determined in a hot stage melting point apparatus model Leica GALEN III. UV λ_{max} of the compound was recorded in PerkinElmer, Lambda-650, UV-Visible Spectrophotometer. The IR spectrum was recorded in PerkinElmer, Spectrum RX-I, spectrophotometer (in the range of 400 to 4000 cm^{-1} wave length with a resolution of $1cm^{-1}$). The elemental analysis was carried out in PerkinElmer 2400 Series II. 1D (^{1}H, ^{13}C and DEPT-135) and 2D NMR (HMBC and HSQC) spectra was recorded (DMSO-d_6) using a Bruker AV-500 MHz NMR spectrometer with Tetramethyl silane (TMS) as internal standard. Precoated TLC plates (TLC Silica gel F254, Merck, Germany) were used in the entire study.

Plant material

Fresh, healthy leaves of *M. edule* were collected from Kolli hills (latitude 10° 12'-11° 7' N, longitude 76°-77° 17' E, above 1000 m MSL elevation), Namakkal districts, Tamil Nadu, India. The nomenclature of collected plant sample was identified by Botanical Survey of India (reference number: BSI/SRC/5/23/2014-15/Tech./248), Coimbatore, Tamil Nadu, India. The voucher specimen of collected sample (specimen number: PU/DBT/NDRL//2010/05) was deposited in Natural Drug Research Laboratory (NDRL), Department of Biotechnology, Periyar University, Salem, India. Collected leaves were washed with tap water, dried at room temperature for three weeks and powdered.

Extract preparation

Pulverized plant material (2 kg) was successively extracted with different solvents (hexane, chloroform, acetone, ethyl acetate and methanol) in increasing polarity manner in a Soxhlet apparatus until the efflux solvent became colourless. All extractives were passed through whatman (No.1) filter paper and concentrated under reduced pressure at 40ºC to yield crude extracts which was stored at 4ºC until use.

DPPH radical scavenging activity guided isolation of antioxidant compound

Based on the preliminary antioxidant activity results,[15] ethyl acetate extract was selected for isolation of pure antioxidant compound by DPPH activity guided isolation method. The ethyl acetate extract (50 g) was fractionated using column chromatography method in increasing polarity (10%) manner using hexane: ethyl acetate (100:0 to 0:100) which gave 51 fractions. Out of 51 fractions, five fractions (F21, F30, F34, F39 and F44) were showing significant antiradical activity. Among them, fraction F44 (hexane: ethyl acetate 20:80) showed very low IC_{50} value (47.33 µg/ml) (Table 1). Hence, the fraction F44 (1.23 g, greenish yellow color, solid in nature) was selected and further purified by silica gel column eluted with hexane: ethyl acetate (100:0 to 0:100) followed by ethyl acetate: methanol (100:0 to 10:90) by increasing the polarity by 5% to yield 21 subfractions. All subfractions were kept at room temperature for allowing condensation and named as MEA1, MEA2, MEA3, etc., (*M. edule* Antioxidant) and tested for its antioxidant potential assays. Significant DPPH radical inhibition was observed in

Table 1: DPPH radical scavenging activity of column fractions from ethyl acetate extract of *M. edule*

Fraction no	IC_{50} (µg/ml)*
F21	66.67 ± 3.21[e]
F30	78.93 ± 0.90[b]
F34	53.33 ± 1.53[d]
F39	56.67 ± 1.53[d]
F44	47.33 ± 0.58[c]
MEA8	45.50 ± 0.50[c]
MEA13	43.57 ± 0.56[b]
MEA14	44.33 ± 1.15[c]
MEA19	32.67 ± 0.58[a]

*-The values are mean of triplicates with standard deviation (mean ± S.D; n =3). Different superscript letters (a-e) in column within treatments indicates significant differences (at $p < 0.05$) when subject to Tukey's multiple comparison test.

4 fractions (MEA8, MEA13, MEA14 and MEA19). Among them, MEA19 fraction (ethyl acetate: methanol 75:25) harbored lowest IC_{50} value (32.67 µg/ml) (Table 1) and single spot was noticed in TLC analysis. Thus, MEA19 (520 mg) fraction was selected for further study and renamed as MEA (*M. edule* antioxidant compound). The MEA compound was subjected to spectral studies such as UV, FT-IR, LC-MS, CHNS analysis, 1D and 2D NMR to predict its structure.

Antioxidant activity

Antioxidant activity of isolated compound on various free radicals were carried out as per the standard protocols *i.e.*, DPPH,[16] nitric oxide,[17] hydroxyl,[18] superoxide[19] radical scavenging activity, and FRAP (ferric reducing antioxidant power assay).[20] Ascorbic acid and butylated hydroxyanisole (BHA) were served as standard reference compounds for all *in vitro* antioxidant assays.

Statistical analyses

All the experiments were carried out at least in triplicates. Data were represented as mean ± standard deviation of three determinations. The Inhibitory Concentrations (IC_{50}) were determined graphically from the curve fitted (non-linear regression) to the mean values of the quotients. The analyses were performed by logarithmically transforming the data to comply with analysis of variance (ANOVA) in a completely randomized design and Tukey's multiple range test (at $p < 0.05$) by employing the statistical software (SPSS 16.0).

RESULTS AND DISCUSSION

Identification and characterization of isolated compound

Spectral data

Pale greenish white colour powder, m.p. 242-243°C; UV (Methanol) λ_{max} nm (log ε) 259 (3.65) and 359 (3.08); IR υ_{max} cm^{-1} (KBr): 3298 (OH), 2920 (-CH-), 1662 (C=O), 1614, 1555, 1512 (-C=C-), 1358, 1318, 1246, 1211, (-C-O- bend), 1167, 1093 (-C-O- stretch), 999, 932, 883, 789, 704, 638, 597 (-C-H out of plane bending); LC-ESIMS *m/z* (% intensity): 611 (89.95) $[M^+ + 1]$; CHNS analysis: C=53.24%, H=4.86%, N=0%, S=0%, O=41.90% (Calcd for $C_{27}H_{30}O_{16}$, 610, degree of unsaturation is 11). The proton and carbon NMR spectroscopy results revealed 30 protons and 27 carbons present in isolated compound (Table 2). The DEPT-135 spectrum used to differentiate the primary, secondary and tertiary carbon signals, but not the quaternary and carbonyl carbons (Table 2). The appearance of 17 carbon (hydrogen containing carbons) signals at δ_C value 122.04, 116.72, 115.68, 101.63, 101.20, 99.13, 94.04, 76.89, 76.35, 74.52, 72.29, 71.01, 70.83, 70.45, 68.70, 67.45 and 18.19 ppm is interpreted as primary, secondary and tertiary carbons. The disappeared carbon (10) signals at δ_C 177.83 (for ketone group), 164.52, 161.68, 157.07, 156.88, 148.86, 145.20, 133.76, 122.05 and 104.43 ppm (for *tert*–carbons) were carbonyl and quaternary carbon signals of the compound. The rhamnose unit containing methyl group appeared above the plane of up field at δ_C 18.19 and glucose moiety containing methlene group was noticed at below the plane at δ_C 67.45 ppm. Above the plane aliphatic CH carbon signals were noticed at 101.63, 101.20, 76.89, 76.35, 74.52, 72.29, 71.01, 70.82, 70.45 and 68.70 ppm. The aromatic CH signals were observed at 122.04, 116.72, 115.68, 99.13 and 94.04 ppm.

Proton or hydroxyl groups attached carbons and weak proton-carbon coupling with nearest carbon skeletons were confirmed by HMBC spectrum (Table 2). The 6-H at δ_H 6.200-6.204 correlated with C-5 at δ_C 161.68, C-7 at δ_C 164.51, C-8 at δ_C 94.04 and C-10 at 104.43 ppm. The 8th proton (δ_H 6.392-6.396) correlated with C-6 at δ_C 99.13, C-7 at δ_C 164.51, C-9 at δ_C 157.07 and C-10 at δ_C 104.43 ppm. The H-2' proton (δ_H 7.540) coupled with C-1' at δ_C 122.05, C-2' at δ_C 116.73, C-3' at δ_C 145.20, C-4' at δ_C 148.86 and C-2 at δ_C 156.88 ppm. These correlations confirmed that pyrone ring attached with substituted phenyl ring (i.e., B-ring) through 2 positions. The 5'-H at δ_H 6.841- 5.858 coupled with C-3' δ_C at 145.20, C-4' at 148.86 and C-6' at δ_C 121.64 ppm. The 6' position proton (δ_H 7.555-7.559) coupled with C-4' at δ_C 148.86 and C-6' at δ_C 121.64 ppm.

Furthermore, C-ring aromatic hydroxyl group 5-OH (δ_{OH} 12.602) was coupled with C-5 at δ_C 161.68, C-6 at δ_C 99.13 and C-10 at δ_C 104.43 ppm. Moreover, rhamnose unit 1''' proton (δ_H 4.353) was coupled with C-1''' at δ_C 101.20, C-2''' (in rhamnose unit) at δ_C 71.02 and C-6'' (in glucose unit) at 67.45 ppm. The correlation of 1''' proton carbon coupling confirmed that rhamnose unit 1''' position attached with 6'' position of glucose moiety. The 6''' H at δ_H 0.992 - 1.004

Table 2: 1D and 2D-NMR data of rutin in DMSO-d_6 at 500 MHz

Position	1H NMR* δ multiplicity, J (ppm)	^{13}C NMR δ (ppm)	DEPT-135 δ (ppm)	HMBC (H→C)	HSQC (H→C)
1	-	-	-	-	**-**
2	-	156.88	-	-	**-**
3	-	133.76	-	-	**-**
4	-	177.83	-	-	**-**
5	-	161.68	-	-	**-**
6	6.20 – 6.204 (1H, d, J = 2 Hz)	99.13	99.13	C-5, C-7, C-8, C-10	H-6 & C-6
7	-	164.52	-	-	-
8	6.392 – 6.396, (1H, d, J = 2 Hz)	94.04	94.04	C-6, C-7, C-9, C-10	H-8 & C-8
9	-	157.07	-	-	-
10	-	104.43	-	-	-
1'	-	122.05	-	-	-
2'	7.540 (1H, s)	116.73	116.72	C-1', C-2', C-3', C-4', C-2	H-2' & C-2'
3'	-	145.20	-	-	-
4'	-	148.86	-	-	-
5'	6.841–6.858 (1H, d, J= 8.5 Hz)	115.69	115.68	C-3', C-4', C-6'	H-5' & C-5'
6'	7.555-7.559 (1H, d, J= 2 Hz)	121.64	122.04	C-4', C-6'	H-6' & C-6'
1"	5.352–5.359 (1H, d, J= 7.5 Hz)	101.64	101.63	-	H-1" & C-
2"	3.221-3.303 (4H, m)	74.53	74.52	-	H-2" & C-2"
3"		76.36	76.35	-	H-3" & C-3"
4"		68.70	68.70	-	H-4" & C-4"
5"		76.90	76.89	-	H-5" & C-5"
6"	3.221-3.303 (1H, m) & 3.703 – 3.724 (1H, d, J= 10.5 Hz)	67.45	67.45	-	H-6" & C-6"
1'"	4.353 (1H, s)	101.20	101.20	C-1'", C-2'", C-6"	H-1'" & C-1'"
2'"	33.221-3.303, (1H, m)	71.02	71.01	-	H-2'" & C-2'"
3'"	3.060–3.078, (1H, m)	70.46	70.45	-	H-3'" & C-3'"
4'"	3.221-3.203 (1H, m)	70.83	70.82	-	H-4'" & C-4'"
5'"	3.060-3.078 (1H, m)	72.30	72.29	-	H-5'" & C-5'"
6'"	0.992 – 1.004 (3H, d, J=6 Hz)	18.19	18.19	C-4'", C-5'", C-6'"	H-6'" & C-6'"
5- OH	12.602	-	-	C-5, C-6, C-10	**-**
7- OH	10.844	-	-	-	**-**
3'- OH	9.678	-	-		**-**
4'- OH	9.184	-	-		**-**
2"-OH	4.391	-	-	-	**-**
3"-OH	4.392	-	-	-	**-**
4"-OH	4.532	-	-	-	**-**
2'"-OH	5.116	**-**	**-**	**-**	**-**
3'"-OH	5.086	**-**	**-**	**-**	**-**
4'"-OH	5.292	**-**	**-**	**-**	**-**

s=singlet; d=doublet; m=multiplet

(methyl group of rhamnose unit) correlated with C-4'" at δ_C at 70.83, C-5'" at δ_C 72.30 and C-6'" at δ_C 18.19 ppm. The overall results of HMBC correlations of isolated compound were depicted in Figure 1 (a).

The direct C-H correlation of the isolated compound was confirmed by the results of HSQC spectrum (Table 2). The (6-H) doublet peak at δ_H 6.200-6.204 coupled with δ_C at 99.13 (C-6) ppm. The 8th proton δ_H at 6.399-6.396 show cross peak correlation with δ_C at 94.04 (C-8) ppm.

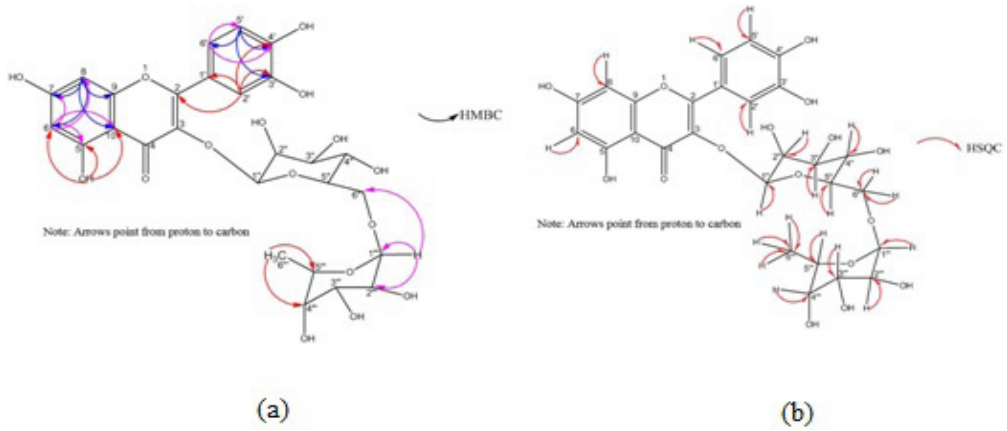

Figure 1: The key HMBC (a) and HSQC (b) correlations of isolated Rutin

Similarly, 2'-H at δH 7.540 was coordinated with δC at 116.73 (C-2'); 5'-H at 6.841-6.858 coupled with δC at 115.69 (C-5') and 6'-H at δH 7.555-7.559 coupled with δC at 121.64 (C-6') ppm. Moreover, results of HSQC spectral study of glucose and rhamnose unit confirmed that the proton corresponding carbon coupling. The glucose unit 1"-H δH at 5.352-5.359 correlated with δC at 101.64 (C-1"); 2"-H at 3.221-3.303 coupled with δC at 74.53 (C-2"); 3"-H 3.221-3.303 connected with δC at 76.36; 4"-H δH at 3.221-3.303 coupled with 68.78 (C-4"), 5"-H δH at 3.221-3.303 associated with δC at 76.90 (C-5") ppm. The 6" position containing two protons appeared in two different environment δH at 3.221-3.303 and 3.703-3.724 coupled with C-6" at δC 67.45 ppm. This cross peak correlation confirmed that the two protons are diasteric protons.

The rhamnose moiety protons 1'"-H δH at 4.353 was cross peak correlated with C-1'" at δC 101.20 ppm. Similarly, 2'"-H at δH 3.221-3.303 coupled with C-2'" at 71.02; H-3'" δH at 3.060-3.078 linked with C-3'" δC at 70.46; 4'"-H δH at 3.221-3.303 associated with C-4'" at 70.83; 5'"-H δH at 3.060-3.078 linked with C-5'" δC at 72.30 and 6'"-H δC at 0.992-1.004 coupled with C-6'" at 18.19 ppm. The overall HSQC correlations are presented in Figure 1(b). Based on the above all spectral analysis (UV, FT-IR, LC-MS, CHNS analysis, ^{1}H, ^{13}C, DEPT-135, HMBC and HSQC) the isolated antioxidant compound was confirmed as Quercetin-3-O-α-L-rhamoside (1→6) -β-D-glucose which is commonly known as Rutin.

Previously, many reports focused on isolation and structural elucidation (through NMR studies) of rutin from various plant parts namely, roots of *Anchusa azurea*,[21] whole plant of *Azolla microphylla*,[22] leaves of *Enterolobium timbouva*,[23] aerial parts of *Phyllanthus amarus*,[24] and leaves and flowers of *Khaya grandifoliola*,[25] etc., which are in good agreement with the results of NMR spectral studies present investigation.

Antioxidant activity of isolated rutin

The results of antioxidant activity of isolated rutin from leaf extract of *M. edule* showed significant scavenging potential on all tested radicals (DPPH, nitric oxide, hydroxyl, super oxide radicals and FRAP assay), in dose dependent manner (Table 3-7). Rutin expressed superior scavenging potential on hydroxyl radicals with lowest IC_{50} value (17.06 µg/ml) which was lower than positive controls (ascorbic acid and BHA).

Moreover, rutin expressed an excellent reduction on Fe^{3+} ions in FRAP analysis with low EC_{50} value (17.29 µg/ml) which was close to the BHA (positive controls). Rutin had

Table 3: Hydroxyl radical scavenging activity of isolated rutin from *M. edule*

Concentration (µg/ml)	Hydroxyl radical scavenging activity (% of inhibition)*		
	Rutin	Ascorbic acid	BHA
20	58.13 ± 0.10[a]	49.60 ± 0.55[a]	42.75 ± 0.52[a]
40	69.26 ± 0.22[b]	61.40 ± 0.41[b]	49.65 ± 0.38[b]
60	74.95 ± 0.14[c]	76.43 ± 0.34[c]	60.18 ± 0.75[c]
80	80.49 ± 0.16[d]	88.25 ± 0.42[d]	73.15 ± 0.38[d]
100	82.79 ± 0.20[e]	94.06 ± 0.60[e]	88.55 ± 0.57[e]
IC_{50}	17.06 ± 0.17	20.47 ± 0.86	40.78 ± 0.98

*-The values are mean of triplicates with standard deviation (mean ± S.D; n =3). Different superscript letters (a-e) in column within treatments indicates significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test.

Table 4: FRAP activity of isolated rutin from *M. edule*

Concentration (µg/ml)	FRAP (OD Values)*		
	Rutin	Ascorbic acid	BHA
20	0.577±0.006[a]	0.305 ± 0.007[a]	0.885 ± 0.007[a]
40	0.970±0.000[b]	0.593 ± 0.006[b]	1.360 ± 0.006[b]
60	1.270±0.010[c]	0.988 ± 0.010[c]	2.133 ± 0.011[c]
80	1.530±0.010[d]	1.505 ± 0.040[d]	2.510 ± 0.009[d]
100	1.673±0.006[e]	2.134 ± 0.012[e]	2.993 ± 0.005[e]
EC_{50}	17.29 ± 0.03	33.62 ± 0.61	11.24 ± 0.37

*-The values are mean of triplicates with standard deviation (mean ± S.D; n =3). Different superscript letters (a-e) in column within treatments indicates significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test.

Table 5: Superoxide radical scavenging activity of isolated rutin from *M. edule*

Concentration (µg/ml)	Superoxide radical scavenging activity (% of inhibition)*		
	Rutin	Ascorbic acid	BHA
20	39.21 ± 0.82[a]	25.31 ± 0.29[a]	13.04 ± 0.62[a]
40	57.44 ± 0.33[b]	34.24 ± 0.42[b]	24.10 ± 0.45[b]
60	65.21 ± 0.16[c]	44.62 ± 0.54[c]	37.28 ± 0.17[c]
80	75.24 ± 0.43[d]	60.06 ± 0.50[d]	40.18 ± 0.42[d]
100	81.61 ± 0.25[e]	75.56 ± 0.25[e]	56.35 ± 0.29[e]
IC_{50}	31.85 ± 0.40	67.03 ± 0.80	90.84 ± 1.76

*-The values are mean of triplicates with standard deviation (mean ± S.D; n =3). Different superscript letters (a-e) in column within treatments indicates significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test.

Table 6: DPPH radical scavenging activity of isolated rutin from *M. edule*

Concentration (µg/ml)	DPPH radical scavenging activity (% of inhibition)*		
	Rutin	Ascorbic acid	BHA
20	42.02 ± 1.07[a]	53.22 ± 0.27[a]	65.42 ± 0.44[a]
40	54.24 ± 0.46[b]	61.12 ± 0.66[b]	69.77 ± 0.58[b]
60	62.63 ± 0.61[c]	73.68 ± 0.38[c]	74.08 ± 0.38[c]
80	71.67 ± 0.69[d]	79.80 ± 0.27[d]	79.94 ± 0.17[d]
100	80.96 ± 0.09[e]	83.53 ± 0.41[e]	84.73 ± 0.38[e]
IC_{50}	32.99 ± 0.46	18.77 ± 0.52	15.48 ± 0.61

*-The values are mean of triplicates with standard deviation (mean ± S.D; n =3). Different superscript letters (a-e) in column within treatments indicates significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test.

Table 7: Nitric oxide radical scavenging activity of isolated rutin from *M. edule*

Concentration (µg/ml)	Nitric oxide radical scavenging activity (% of inhibition)*		
	Rutin	Ascorbic acid	BHA
20	32.37 ± 0.25[a]	28.80 ± 1.21[a]	30.26 ± 0.66[a]
40	43.98 ± 0.11[b]	54.53 ± 0.91[b]	41.23 ± 1.54[b]
60	51.29 ± 0.35[c]	65.79 ± 0.96[c]	53.58 ± 0.67[c]
80	63.26 ± 0.08[d]	79.39 ± 1.22[d]	65.35 ± 1.54[d]
100	72.88 ± 0.14[e]	94.15 ± 1.13[e]	87.28 ± 0.38[e]
IC_{50}	57.34 ± 0.41	36.43 ± 0.71	53.90 ± 0.64

*-The values are mean of triplicates with standard deviation (mean ± S.D; n =3). Different superscript letters (a-e) in column within treatments indicates significant differences (at $p<0.05$) when subject to Tukey's multiple comparison test.

significant inhibitory activity on superoxide radicals with notable IC_{50} values (31.85 µg/ml) which was twofold lower than reference compounds. Considerable DPPH radical quenching rate was observed in rutin with the IC_{50} value of 30.17 µg/ml, which was quite comparable with standards. The sustainable antiradical potential of nitric oxide was found in rutin with high IC_{50} value (30.17 µg/ml). The present findings on the DPPH radical scavenging activity of rutin was supported by earlier reports.[22,26-30] Similarly, many reports also describe the significant antioxidant potential

of rutin on various types of radicals such as TBARS, ABTS, H_2O_2 and FRAP which strengthen the present findings.[22] Rutin previously reported to possesses various biological properties like, anticancer,[31] cytotoxicity,[23] anti-inflammatory,[32] anti-hepatotoxicity,[33] anti-ulcer,[34] and anti-allergic,[35] which are may be due to its antioxidant potential.

CONCLUSION

DPPH scavenging activity guided isolation of antioxidant compound from the ethyl acetated leaf extract of *M. edule* resulted the isolation of rutin. The results of antioxidant potential of rutin show strong antiradical potential on all tested radicles. Hence, the present study strongly suggests that the rutin may be used as a natural antioxidant in order to replace the synthetic antioxidants due to their proved antioxidant potentials.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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