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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT POTENTIAL OF *MEMECYLON UMBELLATUM* BURM LEAF EXTRACTS

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ABSTRACT

Objective: Different dry extracts of *Memecylon umbellatum* Burm leaf obtained by various solvents such as petroleum ether, chloroform, ethyl acetate, acetone, methanol and chloroform water (IP) was screened to reap the benefits of its antioxidant and free radical scavenging properties using ascorbic acid as standard antioxidants. **Methods:** The *in vitro* free radical scavenging activity was evaluated using diphenyl picryl hydrazyl (DPPH) radical method using various concentrations of dry extract in distilled water (1, 2, 4, 8, 16, 20 µg/ml) against blank with ascorbic acid as a standard in same concentrations. **Results:** Among the all extracts, Methanol leaf extract has showed higher Antioxidant activity (84.65 ± 0.064 %) having IC_{50} Value 11.81 ± 0.033 µg/ml at 20 µg/ml. While, IC_{50} value for ascorbic acid was found to be 8.91 ± 0.084 µg/ml. **Conclusion:** The results clearly indicate that Methanol leaf extract of *Memecylon umbellatum* is effective in free radical scavenging. So in future, this may emerge as promising natural herbal source of powerful antioxidant.

Keywords: *Memecylon umbellatum*, DPPH reagent, Antioxidant activity, Ascorbic acid, IC_{50}.

1 INTRODUCTION

The mammalian body has its own multifarious defense mechanism involving natural enzymatic (superoxide dismutase, catalase and glutathione peroxidase) and non-enzymatic (thioredoxin, thiols, and disulfide-bonding) antioxidants which counteract the harmful effects of free radicals and other oxidants[1]. In normal metabolism, the levels of oxidants (i.e. free radicals) and antioxidants in humans are maintained in balance, which is necessary for sustaining optimal physiological conditions[2]. Free radicals are generated as a result of impaired balance between reactive oxygen species (ROS) production and antioxidant enzymes[3]. These are chemically unstable atoms or molecules that cause extensive damage to cells, causes damage to DNA molecule, lipids and proteins[4]. If free radicals overcome the body's ability to regulate them, a condition known as oxidative stress ensues[5]. This could leads to number of life threatening diseases like cardiovascular disease[6], Parkinson's disease[7], cancer[8], mild cognitive impairment[9], neural disorders[10], Alzheimer's disease[11], ulcerative colitis[12], aging[13], diabetes mellitus[14], anaemia[15], atherosclerosis[16], asthma[17]. Protection against this type of disease can be enhanced by ample intake of various dietary food supplements (containing α-tocopherol, b-carotene, and ascorbic acid etc) and synthetic antioxidants, but these synthetic antioxidant capsules and dietary supplements are found to be less effective in various cases. This has attracted a great deal of research interest in natural antioxidants. Several herbs and spices including *Ocimum sanctum, Cichorium intybus, Piper cubeba, Punica granatum, Allium sativum, Delonix regia, Terminalia chebula, Zingiber officinale etc* have been reported to exhibit antioxidant activity[18]. The majority of the antioxidant activity is due to flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins[19, 20].

Memecylon umbellatum Burm. (Family: Melastomataceae) is a small evergreen shrub or tree grows up to 8-14 m tall having young tree branches and bears numerous umbellate cymes. The plant is known as "Anjani" in Sanskrit and "Ironwood tree" in English. Plants are distributed mostly in coastal regions of the Deccan peninsula, the eastern and southern part of India all along the Western Ghats and in the Andaman islands[21, 22]. The leaves have been reported to possess astringent properties and are administered to treat leucorrhoea and gonorrhea[23]. Different extracts of *Memecylon umbellatum* Burm Inflorescences[24] and bark[25] have been evaluated for its antimicrobial potential. Estimation of total content of tannin[26] and Seasonal Variation of Tannin Content in Different Parts has also been carried out[27]. Estimation of Sugars and minerals in healthy and infected parts has also been carried out[28]. Different root extract have been proved to posess antioxidant activity[29]. The decoction of the root is used in the treatment of excessive menstrual discharge[30]. Leaves are also reported to possess antiviral activity[31]. The literature survey reveals that the leaves and roots of Memecylon umbellatum have been investigated for its hypoglycemic activity using alloxan induced hyperglycemia Wistar albino rats[32, 33]. Wound healing activity of ethanolic extract of the leaves has also been reported[34]. Plant contains a wide variety of phytoconstituents such as umbellactone, β-amyrine, Oleanolic acid, ursolic acid, sitosterol and organic acids[35, 36].

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2 MATERIAL AND METHODS

2.1 Plant material

The leaves of *Memecylon umbellatum* were collected in the month of March-April from Gaganbavda hills region, Maharashtra, India. The plant material was taxonomically identified by Dr. S. R. Yadav, Department of Botany, Shivaji University, Kolhapur, India (M.S.). The voucher herbarium specimen is deposited in the Department of Pharmacognosy, Bharati Vidyapeeth College of Pharmacy, Kolhapur.

2.2 Chemical

DPPH Reagent, Ascorbic acid (ACME Chemicals, Mumbai), All other chemicals are of analytical grade and procured from Loba Chem.

2.3 Methods

A standard curve was obtained using Ascorbic acid with the help of double beam UV/Visible spectrophotometer (Jasco-V-630).

2.4 Preparation of extract

Leaves were sorted for foreign matter and dried under shade by spreading in thin layers using aluminum trays for 10 days. Electric grinder (Bajaj-make) was used for powdering soft tissues of leaves. Coarse powder of leaves (#40) was used for extraction.

2.5 Soxhlet extraction process

Extraction was carried out by standard procedure[37-39].One kg powder of roots of Memecylon umbellatum was used for extraction. Sample powder was packed gently in previously washed and dried cloth bag and solvent was placed from the top with the help of funnel to moisten the drug sample. 3.5 liter of solvent (ethyl acetate, methanol, chloroform water, chloroform, and petroleum ether) was placed in distillation flask and assembly was made air tight with sealing wax. Solvents were selected on the basis of extractive values and with their increasing order of polarity. Extraction was carried out at or slightly above the boiling point of each solvent. Extraction was carried out for 18 hours or on the basis of clarity of dropping solvent (saturation). The solvent was collected every time after completion of the process and powder was dried in hot air oven for 24h at 450C. The process was repeated for all the next solvents and finally the dried powder was macerated with 3.5 liter of chloroform water IP (0.25% v/v) at room temperature with frequent shaking. All the liquid extracts were subjected for physical analysis and are concentrated in a rotary film vacuum evaporator (Dolphin, Mumbai) and finally dried under reduced pressure. The residue was weighed, % yield was calculated. All the extracts were further dried over anhydrous calcium chloride and preserved in vacuum desiccators for further studies. Different extracts were abbreviated according to solvent and part of the plant and used throughout the work.

2.6 Physical evaluation of different liquid extracts of leaf

All the extracts were studied for physical evaluation with respect to color, pH, florescence, density, specific gravity, viscosity along with nature of solid residue obtained after concentration of the extracts with % yield has shown in Table 1.

Table 1: Physical analysis of different liquid leaf extracts of *Memecylon umbellatum*

Name of extract	Extract color	pH	Fluorescence			Specific gravity	Density	Viscosity	yield of solids (g)	Nature of solid extract
			D	S	L					
PEEL	DG	6.8	R	O	DB	0.6908	0.8231	0.6124	8.810	Waxy
ChEL	G	6.2	G	R	YG	1.2990	1.5480	1.0290	2.062	Lumpy
SEEL	YG	6.5	Y	P	G	0.6582	0.7850	0.3474	0.375	Powder
EAEL	RB	5.4	-	R	BR	0.6437	0.7265	1.8547	3.065	Powder
ButEL	WR	5.8	-	MG	-	0.6760	0.8058	2.5793	4.604	Powder
AEL	BR	7.2	Y	MW	-	0.6994	0.7801	0.4835	2.243	Powder
EthEL	RB	7.1	-	G	-	0.7902	0.8185	0.9625	8.544	Powder
MEL	RB	6.0	BR	DG	YW	0.8240	0.8848	0.8556	28.36	Waxy
AqEL	RB	5.2	YG	B	G	1.0080	1.0124	1.0210	15.72	Powder

PEEL-Petroleum Ether extract leaf, ChEL-Chloroform Extract Leaf, SEEL- Solvent Ether Extract Leaf, EAEL Ethyl Acetate Extract Leaf, ButEL - n-Butanol Extract Leaf, AEL- Acetone Extract Leaf, EthEL-Ethanol Extract Leaf, MEL-Methanol Extract Leaf, AqEL-Aqueous Extract Leaf, DG -Dark Green, G-Green, YG – Yellowish Green, RB-Reddish Brown, WR-Wine Red, BR-Brownish Red, RB-Reddish Brown, R-Red, G-Green, MG-Milky Green, MW-Milky White, B-Blue, DB-Dark Blue, YW- Yellowish White.

2.7 Phytochemical screening

About 500 mg of each dried extract was dissolved in 100 ml of respective solvent and solution obtained was subjected for Phytochemical screening using different specific and general reagents. Samples were prepared as per the requirement of procedure and tests were repeated for final confirmation of phytoconstituents. The positive phytoconstituents present in different parts with various solvents have shown in Table 2.

Table 2: Phytochemical screening of leaf extracts of *Memecylon umbellatum*

Extract	Sugars		Alk.	Tannins		Glycosides					steroids		proteins	Org. acids		
	R	NR		HT	CT	a	c	s	f	co	ST	TT		C	O	T
AqEL	+	+	-	+	-	-	+	+	+	+	-	-	+	+	+	-
MEL	+	+	-	+	+	-	+	+	+	+	+	-	+	+	+	+
EthEL	+	+	-	+	+	+	-	+	+	-	-	-	+	+	+	+
ButEL	+	+	-	+	+	-	+	-	+	-	+	-	+	+	+	+
AEL	+	+	-	+	+	+	+	+	+	-	+	-	+	+	+	+
EAEL	+	+	-	+	+	+	-	-	+	-	-	+	+	+	+	-
ChEL	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
SEEL	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
PEEL	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-

AqEL-Aqueous Extract Leaf, MEL-Methanol Extract Leaf, EthEL-Ethanol Extract Leaf, ButEL-n Butanol Extract Leaf, AEL-Acetone Extract Leaf, EAEL-Ethyl Acetate Extract Leaf, ChEL-Chloroform Extract Leaf, SEEL-Solvent Ether Extract Leaf, PEEL-Petroleum Ether Extract Leaf, Alk-Alkaloids, Gly-Glycosides, Org.acids-Organic acids, R-Reducing sugars, NR-Non Reducing sugars, HT-Hydrolysable Tannins, CT-Condensed Tannins, a-anthracene glycosides, c-Cardiac glycosides, s-Saponin glycosides, f-Flavanoidal glycosides, co-Coumarin glycosides, ST-Sesquiterpene, TT- Triterpene, + Positive, - Negative.

2.8 Screening of extracts for in- vitro antioxidant activity using DPPH Assay[40-46]

Extracts showing presence of triterpenes and polyphenolic compounds were screened for antioxidant activity using DPPH reagent. DPPH assay is most widely used method for determination antioxidant potential. Its use has been previously reported for species *Acacia caesia*[47], *Aerva Lanata*[48] etc.

2.8.1 Reagents for antioxidant activity

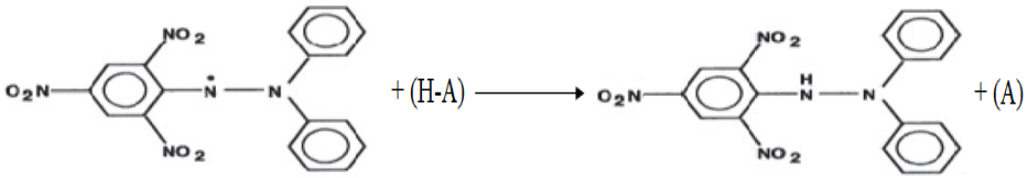
1. DPPH Reagent: Methanolic solution of DPPH (0.1 mM): 39.4 mg of DPPH was dissolved in one liter of analytical grade methanol.

2. Standard solution: Ascorbic acid was used as standard in following concentrations 1, 2, 4, 8, 16, 20 µg/ml in methanol.

3. Sample preparation: Test samples of each dry extract were prepared by dissolving in distilled water in the various concentrations as 1, 2, 4, 8, 16, 20 µg/ml.

2.8.2 Principle:

The scavenging reaction between (DPPH) and an antioxidant (H-A) can be written as:



Free radical (Purple) Non radical (Yellow)

DPPH (1, 1-diphenyl-2-picrylhydrazyl) is a stable free radical, characterised by the delocalisation of the spare electron over the molecule as a whole. So this does not dimerize unlike the other free radicals. The delocalization of electron also gives rise to the deep violet color. When antioxidants react with DPPH, which is a stable free radical is reduced to the DPPHH i.e. 1 - 1 diphenyl - 2 – picryl hydrazine and as consequence there is loss of this violet color. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability.

2.8.3 Procedure

The DPPH scavenging activity was performed using a solution of 0.1 mM DPPH in methanol solution and 1.0 ml solution was added in 3.0 ml of test samples of each dry extract having concentrations as 1, 2, 4, 8, 16 and 20 µg/ml in methanol and kept in darkness. Thirty minutes later, the absorbance was measured at 517 nm. A blank was prepared without adding the extract. Ascorbic acid at concentration 1, 2, 4, 8, 16, 20 µg/ml was used as standard. Lower the absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical was calculated using the following equation.

$$\text{DPPH Scavenged (\%)} = \frac{A_{\text{Control}} - A_{\text{Test}}}{A_{\text{Control}}} \times 100$$

Where 'A control' is the absorbance of the control reaction and 'A test' is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the different extract was expressed in % DPPH radical scavenged and the results are given in table 3.

Table 3: Antioxidant activity and IC_{50} value of different extracts of *Memecylon umbellatum

Sr. No.	Solvent	% Antioxidant activity of different extracts at various concentrations (µg/ml)						IC_{50} (µg/ml)
		1	2	4	8	16	20	
1	Standard	12.36 ± 0.035	29.27 ± 0.055	37.42 ± 0.314	44.89 ± 0.121	74.92 ± 0.099	91.90 ± 0.054	8.91 ± 0.054
2	Petroleum ether	0.36 ± 0.248	0.87 ± 0.159	1.48 ± 0.097	2.39 ± 0.342	2.88 ± 0.463	3.17 ± 0.084	315.45 ± 0.059
3	Chloroform	0.29 ± 0.111	1.08 ± 0.058	1.97 ± 0.096	2.98 ± 0.278	3.86 ± 0.396	4.76 ± 0.036	210.08 ± 0.063
4	Ethyl acetate	8.32 ± 0.296	22.96 ± 0.398	41.36 ± 0.179	52.69 ± 0.224	63.89 ± 0.419	77.24 ± 0.059	12.94 ± 0.016
5	Acetone	11.23 ± 0.385	31.69 ± 0.054	47.65 ± 0.158	57.38 ± 0.269	68.93 ± 0.114	78.83 ± 0.167	12.68 ± 0.064
6	n-Butanol	14.39 ± 0.159	38.96 ± 0.178	48.36 ± 0.342	61.32 ± 0.329	71.88 ± 0.152	82.01 ± 0.018	12.19 ± 0.089
7	Ethanol	5.07 ± 0.118	18.65 ± 0.159	26.65 ± 0.277	33.33 ± 0.096	39.37 ± 0.518	49.20 ± 0.068	20.33 ± 0.018
8	Methanol**	9.56 ± 0.278	19.63 ± 0.152	45.69 ± 0.329	59.36 ± 0.114	79.69 ± 0.278	84.65 ± 0.064	11.81 ± 0.033
9	Aqueous	2.12 ± 0.114	4.48 ± 0.096	6.65 ± 0.342	7.08 ± 0.196	9.96 ± 0.059	11.11 ± 0.128	90.01 ± 0.055

** Indicates ± SD (n=5) ** indicates more potent extract & significance ($p<0.05$)*

IC_{50} value was determined to express antioxidant activity. It is the concentration of fractions that inhibits the formation of DPPH radicals by 50%. The lower IC_{50} value represents the higher antioxidant activity of the tested sample.

3 RESULTS AND DISCUSSION

3.1 Evaluation of different liquid extracts of leaf

Most of the extracts have shown different color in different solvents. Some of the extracts have showed typical florescence either in day or short (254nm) and long (366nm) wavelengths. Leaf extract showed maximum pH 7.2 for acetone and minimum pH 5.2 for aqueous extract. Specific gravity was found highest (1.2990) for chloroform extract and lowest (0.6437) for ethyl acetate. Also maximum viscosity (2.5793cp) for n-butanol and minimum (0.3474cp) for solvent ether was observed. The maximum % yield 28.36 for leaf was found for methanol and minimum 0.375 % for solvent ether.

3.2 Phytochemical screening of different extracts

Polar solvents used in the process of extraction have shown the presence of polar constituents such as mono and disaccharides, proteins amino acids different glycosides like anthracene, cardiac, flavanoidal, saponin and coumarin type glycosides. Polyphenols like tannins, organic acids, minerals and triterpenes were also found in most of the polar extracts while non polar solvents showed positive tests for sterols, aglycones of different glycosides, fatty acids and polysaccharides. Phytochemical screening of different liquid extracts showed the presence of reducing, nonreducing sugars, tannins and proteins (both hydrolysable and condensed) in almost every extract except for Chloroform (ChEL), Solvent Ether (SEEL) and Petroleum Ether Extract (PEEL). No traces of alkloids were detected in any of the extract. Chloroform (ChEL), Solvent Ether (SEEL) and Petroleum Ether Extract of leaf (PEEL) shows dearth of different glycosides, while other extract showed existence of cardiac, saponin, flavanoidal and coumarin glycosides. Being nonpolar in nature ChEL, SEEL and PEEL showed presence of Steroids and terpenes. Some extracts shows presence of organic acids also.

3.3 Screening of different extracts for In-vitro antioxidant activity

In the present study, in vitro antioxidant activity of the different leaf extract of *Memecylon umbellatum* was investigated by DPPH radical scavenging assays. It is probably due to the presence of phytochemicals like polyphenolics, steroids, glycosides and saponins, highly responsible secondary metabolite for antioxidant activities in these species. Methanol leaf extract showed 84.65 ± 0.064 % antioxidant activity which is higher than other extract [Pet. Ether (3.17 ± 0.084), Chloroform (4.76 ± 0.036), Ethyl acetate (77.24 ± 0.059), Acetone (78.83 ± 0.167), n-Butanol (82.01 ± 0.188) Ethanol (49.20 ± 0.068) and Aqueous extract (11.11 ± 0.058)] at 20 µg/ml. Pet. Ether, Chloroform and aqueous leaf extracts showed very feeble antioxidant activity. Antioxidant activity of different extracts was found to be in order as follow,

Methanol > n-butanol > Acetone > Ethyl acetate > Ethanol > Aqueous > Chloroform > Pet. Ether.

IC_{50} value, a guide for antioxidant value was determined from % antioxidant activity has been shown in figure 1.

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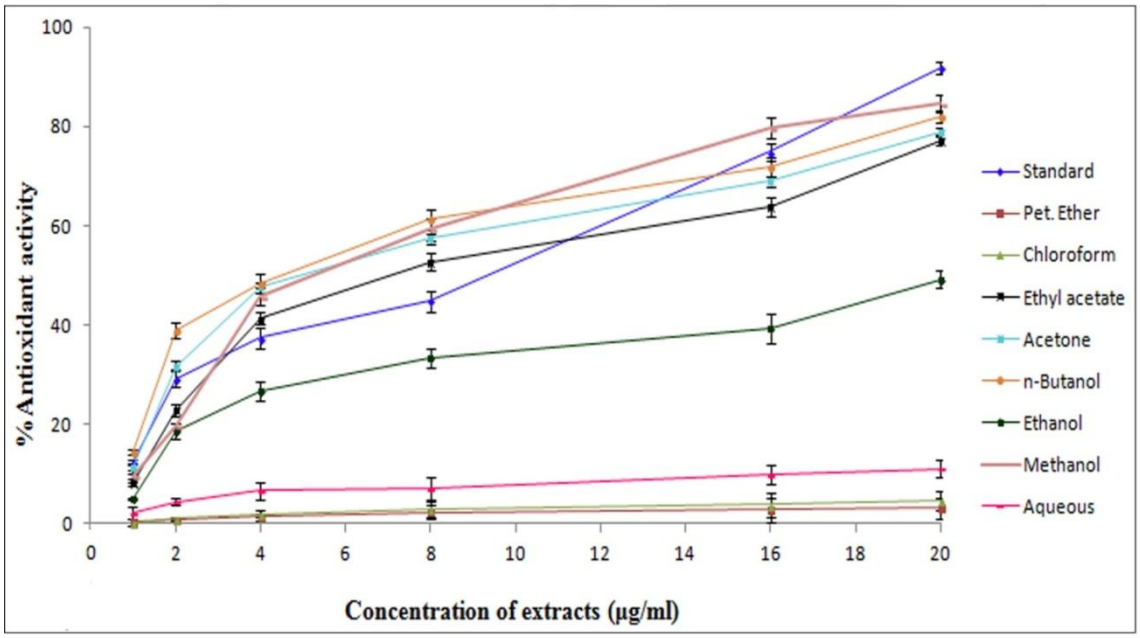

Figure 1: Plot of % antioxidant activity verses various concentrations of standard and different extracts.

Methanol leaf extract showed significant *(p<0.05* Graphpad instat 3*)* IC_{50} value (11.81 µg/ml) compared to standard i.e. ascorbic acid (8.91 µg/ml). All other extracts showed higher IC_{50} value, indicate lesser antioxidant activity than standard and methanol leaf extract. Pet. Ether and chloroform leaf extract showed higher IC_{50} value, 315.45 and 210.08 µg/ml respectively. IC_{50} value of different extract has been shown in figure 2.

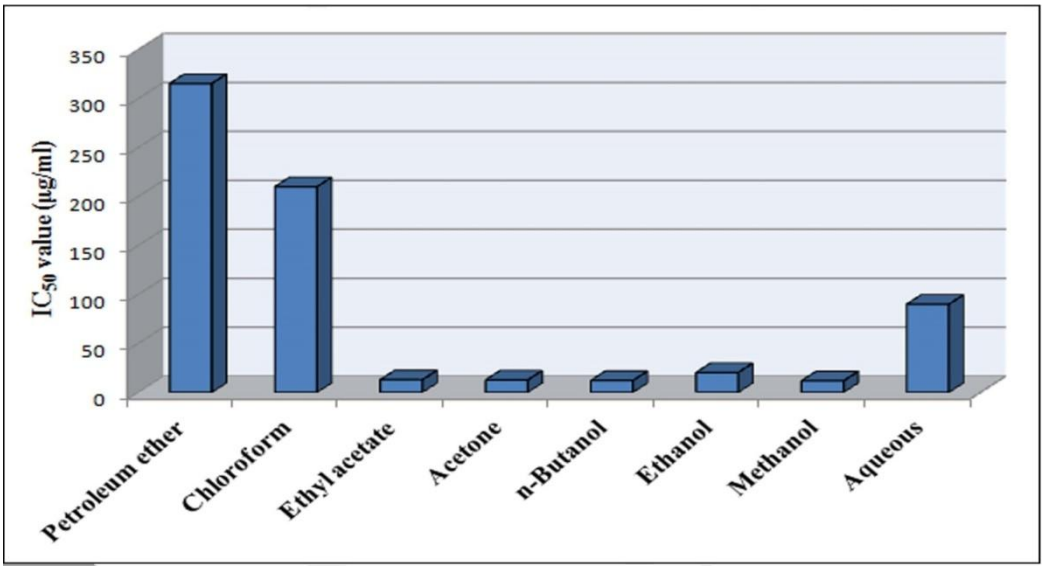

Figure 2: Graph showing IC_{50} value of different extracts of *Memecylon umbellatum* leaf.

4 CONCLUSIONS

From the findings of this study, it can be concluded that *Memecylon umbellatum* leaf extracts, emerging as promising natural herbal sources of antioxidants and can be used in nutritional or pharmaceutical fields for the prevention of free radical-mediated perilous diseases (oxidative stresses). However, in-vivo assays are essential to characterise it as biological antioxidants. In addition to this, flavonoids, mainly responsible for antioxidant activity need to be investigated in details.

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CONFLICT OF INTEREST:

We declare that we have no conflict of interest.

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