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Research Article

Antioxidant activity evaluation of *Memecylon lushingtoni* Fruit

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Abstract: In the present study evaluation of *Memecylon lushingtoni* fruit pulp (*M.l.*Fr.) Aqueous extract has been quantified for antioxidant activity. Total phenolic content (TPC), total flavonoid content (TFC) was evaluated and tested for its antiradical scavenging activity by DPPH and ABTS assays using standard photometric methods. *M.l.*Fr. exhibited prominent values for TPC, TFC and *in-vitro* radical scavenging activity. Our findings concluded that ripe fruits of *Memecylon lushingtoni* contain an appreciable amount of high valued therapeutic compounds and could act as a potential source of natural antioxidants.

Keywords: *Memecylon lushingtoni,* Antioxidant activity, Horsley hills.

Introduction

Antioxidants play a key role in protecting against oxidative damage. Plant extracts has a great potency in playing as natural antioxidants (Edziri *et al.*, 2020). Fruits are vital dietetic components containing various bioactive constituents which have been established to be useful to control and treat various persistent diseases like diabetes, obesity, cancer and cardiovascular diseases (Devalaraja *et al.*, 2011).

Our extensive field survey and enlisting of wild edible plants of seshachalam hills revealed few unexplored plant species where no data is available on its biological compounds (Ganesh and Sudarsanam, 2013; Anjaneyulu and Sudarsanam, 2013). One among the unexplored is *Memecylon lushingtoni*, a rare species which is very closely related to *Memecylon umbellatum* and can be differentiated in flowering and fruiting stage only. *Memecylon lushingtoni* fruits are edible and were swallowed by fauna like birds, squirrels. However, no ethnic information was reported on phytochemistry nor on antioxidant property of this taxon which is lacking and so far not been investigated to the best of our knowledge. In view of exploring of unexplored we intended to investigate the antioxidant potency of *Memecylon lushingtoni* ripe fruits.

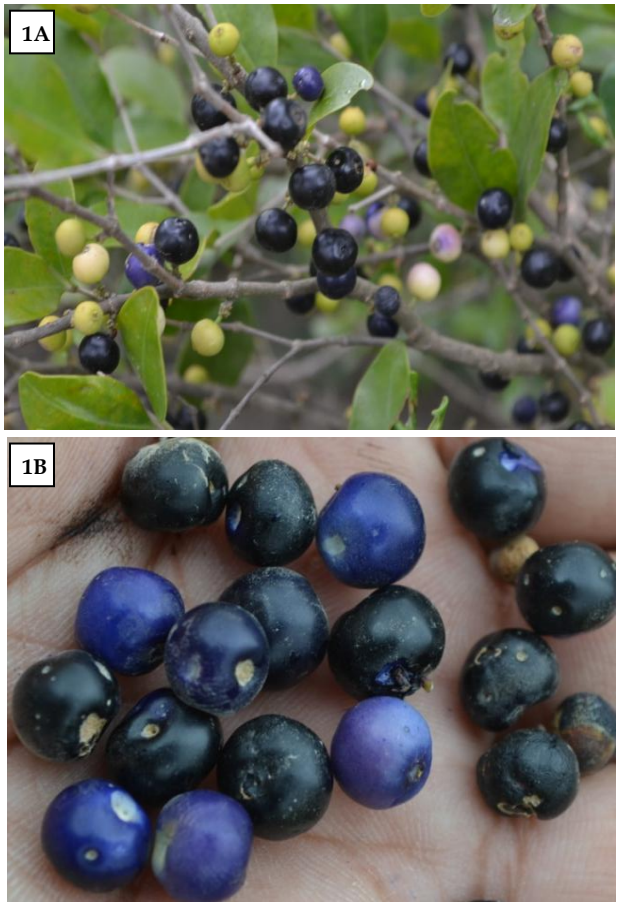

Figure. 1. *Memecylon lushingtoni*
1.A. Immature and Mature fruits.
1B. Ripe fruits.

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Materials and Methods

Sample collection

Fully ripened fresh samples of *Memecylon lushingtoni* (Family : Myrtaceae) were collected from the rocky crevices in North east aspect of Horsley Hills, a hill station in southern eastern ghats of Andhra pradesh (Figure 1.A - B).

Identification of Plant material

Taxonomic identity was done with Local flora (Chetty *et al.,* 2018; Pullaiah *et al.,* 2018) The herb-arium specimen (SVUTY/MYR/4762) was pre-pared according to the method of Jain and Rao (1977) and has been deposited in the Herb-arium of Department of Botany, Sri Venkates-wara university, Tirupati, Andhra Pradesh for further use.

Preparation of the extract

Precisely, 250 g of fresh ripe fruit pulp with peel were squeezed and seed is removed with filtrate and were weighed and extracted with 80% methanol and aqueous distilled water. The homogenized mixture was filtered and used for quantitative estimation (Nath *et al.,* 2013, Mitta *et al.,* 2014).

Estimation of Total phenolic content (TPC)

Folin–Ciocalteu colorimetric method was follo-wed for quantification of Total phenolic content (TPC). TPC expressed as gallic acid equivalents (GAE) in mg/100gm (DW).

Estimation of Total flavonoid content (TFC)

Aluminum chloride ($Alcl_3$) colorimetric method was adapted for quantification of Total flavor-noid content (TFC) and expressed as Quercetin equivalent (QE) in mg/100gm (DW).

Estimation of Phenolics and Flavonoids (Poly-phenols) were evaluated following Singleton (1999).

Total Antioxidant capacity (TAC)

Total antioxidant capacity was determined by Phosphomolybdenum method as suggested by Prieto *et al.,* (1999) and Akhtar *et al.,* (2018). Briefly, 0.1 mL of the sample was mixed with 1 mL of reagent solution (0.6 M. H_2SO_4, 28 mM sodium phosphate and 4 mM Ammonium molybdate). Mixture was incubated at 95°C for 90 min and then cooled to room temperature and the absorbance was measured at 695 nm. TAC of each sample was expressed as ascorbic acid equivalent.

Antioxidant assays-

Radical scaveniging activity

Measurement of the free-radical scavenging activity in percentage of the *M.lushingtoni* fruit extract was evaluated by DPPH and ABTS assay and calculated with the formula given below.

$$\textbf{Inhibition\%} = [(A_B - A_A) / AB)] \times 100$$

A_A = absorbance of *M.l.*Fr. extract . where A_B = absorbance of DPPH solution.

DPPH assay

Determination of DPPH assay was performed as described by Brand-Williams *et al.* (1995) followed by Kumar *et al.,*(2010). In short, 10µL of aqueous extract was added to 100 µL of 0.12mmol/L DPPH (2,2-diphenyl-1-picryl-hydrazyl) radical was prepared by dissolving 25 mg of DPPH in 100 ml methanol and stored at -25°C until use. 5 ml of this stock solution was added to 1 ml of *M. lushingtoni* fruit extract solution at different concentrations (10, 25 and 50 µg/ml). The antioxidant ability was expressed as percent of Inhibition, which represent the concentration of the sample necessary to inhibit DPPH radicals.

ABTS assay

ABTS (2,20-azino-bis (3-ethylbenzo thia-zoline-6-sulfonic) acid) assay was done as described by Re *et al.,*(1999) and followed by Wang *et al.* (2008) with minor modifications. Results were expressed as micromole of Trolox equivalents per gram of dried samples (µmol TE/g). The capability to scavenge the ABTS radicals was calculated in percentage.

Solvents for titrations were used according to the suggestions reported by Bahukhandi *et al.,* (2013) and Suyal *et al.,* (2020) for estimation of radical scavenging activity as antioxidant pote-ncy of the testing material.

Results & Discussion

Quantitative analysis of Polyphenols

Total Flavonoid content (TFC)

Flavonoid contents varied from 17.2mg- 26.2 mg (QE/g) DW (QE – Quercetin equivalent).

Total phenolic content (TPC)
The values of TPC determined in the present study varied to a lesser extent and ranged from 14.98mg - 21.5mg GAE/g DW (GAE - Gallic acid equivalent).

Total Antioxidant capacity (TAC)
The aqueous extract sample exhibited 44.8 mg – 54.2 mg Vit. C eq./g DW. (Vitamin C equivalent).

Antioxidant assays
Free radical scavenging activity
Antioxidant activity of aqueous extracts *M. lushingtoni* ripe fruits was evaluated with simple photometric assay with commercially available stable ABTS and DPPH free radical scavengers to evaluate the ability of biological compounds in terms of percentage of scavenging capacity.

Table : 1: Antiradical scavenging activity (%)

Sam-ple teste d	Conc. (µg/ mL)	(DPPH radicals scavenged) % Inhibition ± SD	(ABTS radicals scavenged) % Inhibition ± SD
Ml.F.	10	11.28±1.32	19.46±0.86
	25	24.43±0.28	25.38±1.04
	50	31.18±0.44	43.21±0.60
BHT	1.0	29.44±0.78	31.16±1.14
	2.5	36.25±0.26	47.08±0.48
	5.0	62.12±0.69	66.42±1.05
Asco rbic acid	1.0	13.52±1.08	15.18±0.84
	2.5	47.21±0.62	32.46±0.88
	5.0	63.31±0.40	64.04±0.47

To evaluate antioxidant activity of aqueous extract, their ability to scavenge DPPH and ABTS radicals were investigated (Table 2). Results showed that aqueous extract had the best antioxidant activity by DPPH and ABTS methods with the (BrandWilliams *et al.,* 1995, Noreen *et al.,*2017).

The percentage DPPH scavenging activity of aqueous fruit extract of *M. lushingtoni* was found to be between 11.28±1.32% at 10 µg/ml to 31.18±0.44% at 50 µg/ml. The scavenging capacities of the extract for the ABTS radical were measured and their percent inhibition values with ABTS assay showed between 19.46±0.86% at 10 µg/ml to 43.21±0.60% at 50 µg/ml. Values are expressed as the mean of triplicates.

Plant secondary metabolites like flavonoids and other phenolic compounds constitute antioxidant capacity (Srivastava, 2018; Edziri *et al.,*2020; Labdelli *et al.,* 2020). In this investigation, fruit extract of *M.lushingtoni* caused the reduction of DPPH, ABTS radicals which caused decolorization according to dose dependency and expressed as percentage antioxidant activity.

The percent deterrence of DPPH radical by the extracts was compared to a known synthetic antioxidant, Butylated Hydroxytoluene (BHT). Colour density shows the antioxidant potency of the extract. (Bhatt and Negi, 2012). Synthetic antioxidant BHT showed % inhibition of 29.44±0.78, 36.25±0.26, 62.12±0.69 at 1.0, 2.5 and 5(µg/mL) against DPPH. Ascorbic acid showed % inhibition of 13.52±1.08, 7.21±0.62, 3.31±0.40 at 1.0, 2.5 and 5(µg/mL) against DPPH. BHT showed % inhibition of 31.16±1.14, 47.08± 0.48, 66.42±1.05 at 1.0, 2.5 and 5(µg/mL) against ABTS. Ascorbic acid showed % inhibition of 15.18±0.84, 32.46±0.88, 64.04±0.47 at 1.0, 2.5 and 5(µg/mL) against ABTS. Estimations were made against standard antioxidant compounds *viz*., Ascorbic acid and BHT. All crude extract (*Ml.F.*) basically reacted with DPPH and BHT and might form free radicals which finally gradually changed the colour of DPPH and BHT (Labdelli *et al.,*2020) ABTS radical scavenging assay involves a method that generates a blue/green ABTS+ chromophore *via* the reaction of ABTS and potassium persulfate (Bhardwaj *et al.,*2016). Our research on ayurvedic drug "Nitryaprasa" revealed interesting findings on neutraceutical household drugs (Bharathamma *et al.,*2015)

In the present experiment, the results thus obtained suggest that this supporting evidence for crude aqueous fruit extract of *M. lushingtoni* posses high valued amount of Phenolic, Flavonoid compounds which are the source of radical scavenging property (Cosme *et al.,* 2020)

and these findings justify that wild edible fruits of *Memecylon lushingtoni* posses therapeutic natural antioxidant properties. However a further biochemical characterization and pharmaceutical investigation are required besides traditional techniques and optimize parameters in order to derive, isolate the novel natural antioxidant compounds or to develop any drugs of natural origin.

Conclusion

In conclusion, our study provided baseline information on biochemical attributes and reported ripe fruits of *Memecylon lushingtoni*, a rare species occurring in Horsley Hills possess antioxidant property and revealed that this could emerge as an effective alternative dietary source of natural antioxidant compounds for traditional medicine, food, nutraceuticals and pharmaceutical industries.

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