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# Bioprospecting *Terminalia muelleri* Benth. for antioxidant activity

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**Abstract**
**Objective:** Use of Myrobalan to heal Human ailments is practiced since Ayurveda, Unani and Siddha Medicines. This has resourced evaluating medicinal properties of species of Genus: *Terminalia* L., globally. Focusing this, Australian Almond- *Terminalia muelleri* Benth. was Bioprospected for Antioxidant potential by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) & Nitric oxide (NO) Free Radical Scavenging Assay.
**Method:** Aqueous ethanolic bark extract (AEBE) of *T. muelleri* (*Tmu* AEBE) was Soxhlet extracted and condensed using Rotovap. Different weighing concentration (5-50 µg/mL) of *Tmu* AEBE were experimented for antioxidant potential, against synthetic antioxidant- Ascorbic acid. DPPH Free Radical Scavenging Activity (FRSA) was experimented by Blois method while NO FRSA was experimented by Nims *et al*. method. Half maximum effective concentration ($EC_{50}$) and its inverse called as Antiradical power (ARP) was also calculated for the first time for *T. muelleri*.
**Results:** FRSA of *Tmu* AEBE obeyed Concentration dependent expression following Non Linear progression. These DPPH & NO FRSA results were expressed as % Free Radical Scavenged (%). NO FRSA results were expressed as Mean ± S. D. of three replicates (n=3) and analyzed statistically using One way ANNOVA. Quantal concentration dependent Radical Scavenging curves were processed for calculating $EC_{50}$ (expressed as µg/mL) using Origin Pro 8.5.
**Conclusion:** Phytoconstituents constituting *Tmu* AEBE constituted to DPPH & NO Free Radical Scavenging Potential, *in-vitro*. Thus these phytoconstituents will be structured using modern analytical instrumentations and their Antioxidant capacities will be Bioprospected, individually.

**Keywords:** Antioxidant activity, antiradical power (ARP), DPPH, $EC_{50}$, NO, *Terminalia muelleri* Benth.

## Introduction
In India, Traditional Ethnic knowledge of healing Humans, by using botanicals, was made known through various Samhita. Since then and even today, phytochemical constituents orchestrating array of biological responses remains unpredictable. Was it a serendipity or a curious traditional knowledge, is still a question. Based to this, botanicals were screened for Secondary metabolites- constituting Class of compounds, followed by *in-vitro* and *in-vivo* Bioassay experiments that ought to validate the Biology behind Healing. This has advented "Bioprospecting", which yet seems to be new to India [1].
Till now only *Terminalia* Arjuna Wight & Arn. is bio prospected for antimicrobials anti-biofilm potential [2]. Further, Coulibaly (2014) bio prospected medicinal plants for antioxidant potential [3].
Science has already correlated Diet & Metabolism induced generation of Free Radicals in on setting Human Diseases. Focusing this, *T. muelleri* Benth. (Combretaceae)- Australian Almond was Bioprospected for Antioxidant activity.

## Taxonomy
*Terminalia muelleri* Benth, *Fl. Austral. 2: 500. 1864.* Type: In Queensland, Australia (Syntype, 05-09-1966, Mueller, F.J.H, 8628 [K- K000786544]).
This species of Genus: *Terminalia* L. [4], Family: Combretaceae, is native of Queensland, Australia. Taxonomically, its identity as *T. muelleri* Benth. was described in their Flora Australiensis [5], under Section: Myrobalanus Gaertn**.** Later Kuntze synonymized it with *Myrobalanus muelleri* (Benth.) Kuntze [Revis. Gen. Pl. 1: 237. 1891] [6].
Habit and Distribution (Figure 1. 1b): This Mueller's Damnson was introduced in Dr. Babasaheb Ambedkar Marathwada University, Department of Botany Botanic Garden, Aurangabad and Beed, Maharashtra, India. Gaikwad *et al.* (2014) added *T. muelleri* Benth. to Marathwada region of Maharashtra [7]. Further, its occurrence is also witnessed from Delhi, Agra and Bangalore states of India (efloraofindia/*Terminalia muelleri*) [8].


**Fig 1:** 1b. Collection site of *T. muelleri* Benth. Aurangabad, (MS) India.

**Biology and Potential value**.

This species of botanical interest is accounted for isolation of [3, 4, 5] trihydroxy benzoic acid that exerted antibacterial effect against strains of *Staphylococcus aureus* & *Escherichia coli* [9]. Nanoparticles synthesized from its leaf extract exert antioxidant activity [*in-vitro* (DPPH FRSA [10, 11]) & *in-vivo*] and anti-inflammatory activity [11]. Additionally, its phenolic glycoside content [12] and DPPH free radical scavenging activity were also concerned with Anti Radical Power [13]. Ellagic acid- a Tannin [14], isolated from *T. muelleri* Benth. was found responsible for eliciting antioxidant activity [11]. Methanolic extract of *T. muelleri* inhibited MCF- 7 Breast cancer cell Line, Hep G2 and HT- 29, with characteristic IC value- 40 µg/ ml [15]. It also inhibited HIV- 1 progression [16].

In recent, this sp. was explored for Inhibitive Acetylcholine Esterase function [17], anti- inflammatory activity assayed through Carragenan induced Lind paw model [18] and antimicrobial [19, 20] activities i.e. Antifungal [21] & antibacterial [22] activities.

**Key concerns**

Essence from flowers of Mueller's Damson is reported to induce Allergic asthmatic reactions [14].

**Methodology**

**1. Collection of *T. muelleri* Benth. Specimens: Flowering/ fruiting twigs, leaf and bark.**

Flowering and fruiting twigs of *T. muelleri* Benth. were collected from Department of Botany Botanic Garden, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (Fig. 1- 1a).

**2. Acquiring Voucher No. from BAMU Herbarium**

A processed specimen mounted on a labeled herbarium sheet, with collection No- SAP & ASD- 02, was submitted to Department of Botany Herbarium, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad (MS), acronymed as BAMU, for obtaining Voucher Number.

**2.1 Chemicals**

2,2-Diphenyl-1-picrylhydrazyl, Nitric oxide, sodium nitropruside, Griess reagent, PBS (1X) and Ascorbic acid were obtained from Sigma-Aldrich (Chem Trade, Aurangabad (MS), India.

**3. Preparation of Aqueous Ethanolic Bark extract (Tmu AEBE) extract**

10 gm of shade dried bark sample was pulverized to fine powder, using a blender. Blended powders were knot tied (knotted) in Whatman's filter paper No. 01 and Soxhlet extracted using 50% aq. ethanol (400 ml, 24 hrs.). Obtained extract was condensed using rotary evaporator ($75^0$C, 60 rev. /min., 0.41 hrs.). On drying *Tmu* AEBE transformed to scarlet red yellowish crystals. These crystalline form was used for measuring Antioxidant activity. Further unused, left, crystalline samples was stored in stoppered bottles and refrigerated for long term purpose.

**4. DPPH Free Radical Scavenging activity (FRSA) of *T. muelleri* Benth**

DPPH Free Radical Scavenging activity of crystallized Bark (*Tmu* AEBE) extract of *T. muelleri* Benth. was determined by method adopted by Blois [23] and Brand- Williams *et al.* [24]. % Inhibition of DPPH Free Radical was deduced using-

- Methodology adopted by Bankeblia [25].
- Calculations followed Marinova & Batchvarov's formula [26].
- Quantal Concentration dependent % DPPH Scavenged curve was processed using software- AAT Bioquest.

**Additionally by using Brand-Williams *et al.* method [24]**

- The reaction mixture effect of Initial DPPH concentration and extract on % of DPPH remaining was evaluated.
- Inverse of $EC_{50}$ i.e. Antiradical Radical Power (ARP) was evaluated.

Different weighing concentrations (5-50 µg/ml) of *Tmu* AEBE crystals were aspirated to ten labeled test- tubes respectively. To each, an Ethanolic DPPH ($_{et}$DPPH) solution was added. Absorbance of formed color complex was recorded at 517 nm on Systronics UV- Vis dual beam spectrophotometer [24]. The Antioxidant activity was expressed as Half maximum Effective concentration ($EC_{50}$) and its value was determined using Quantal concentration dependent % DPPH $_{fr}$ Scavenged curve.

**5. Nitric Oxide (NO) FRSA of *T. muelleri* Benth.**

Nitric oxide (NO) free radical scavenging activity (FRSA) of *Tmu* AEBE was determined by method adopted by Nims *et al.* [27] and modifications attributed by Hazra *et al.*[28]. Respectively 5-50µg/mL concentrations of *Tmu* AEBE & Ascorbic acid were aspirated to 10 ml test tube, each. A test tube devoid of *Tmu* AEBE served as a blank (-ve control i.e. devoid of *Tmu* AEBE). To each test tube 1ml of 10mM of SNP prepared in 1X PBS was added. These test tubes were incubated for 2hrs. To each incubated test tube, 0.5 ml of Griess reagent was added. Absorbance measurements for the formed chromophores (colored diazo products) were recorded at pre specified wavelength- 540nm [27], using a pair of Quartz cuvettes of 1 cm thickness.

% of NO free radical scavenged by *Tmu* AEBE was estimated using formula

$$\% \text{ of NO free radical scavenged} = \frac{Ao - As}{Ao} \times 100$$

Where $A_0$ is the Absorbance of Control and $A_S$ is the Absorbance of Sample.

**Data Analysis**

FRSA of *Tmu* AEBE obeyed Concentration dependent expression following Non Linear expression. Graphs revealing drop in absorbance were generated using AAT Bioquest Software. These DPPH & NO FRSA results were expressed as % Free Radical Scavenged (%). % NO FRSA results were expressed as Mean ± S. D. of three replicates (n=3) and analyzed statistically using One way ANNOVA. Quantal concentration dependent Radical Scavenging curves were processed for calculating ($EC_{50}$ expressed as µg/mL) using Origin Pro 8.5 and considered significant ($P$<0.05).

**Results and Discussions**

**I. *Tmu* AEBE DPPH FRSA**

As presented in Figure 2, % scavenging abilities of *Tmu* AEBE was found to be–

1. Increased, for a concentration in a Time dependent manner (Figure 2-2a).

With increase in Time, DPPH radical scavenged by the extracts increased and thus % of DPPH remaining increased (Fig. 2-2b.). Thus accessed Antioxidant activity evidenced by Time dependent decrease in absorbance value (at 517 nm) of reaction mixture (Table 1) and was in accordance with the results stated by Blois [23].

**2. Increased, by increasing the concentrations of extracts.**

Following Blois [23], Brand-Williams *et al.* [24] and Bankeblia method [25], % DPPH scavenging abilities of *Tmu* AEBE ranged from 06.52% - 43.31% and that of Ascorbic acid ranged from 2.18% - 34.55%.

For dried extract experimented, % DPPH scavenging ability was found increasing with increasing concentration. Thus Graph 2- 2b (Fig. 2) obtained for *T. muelleri*- % DPPH inhibition, is of Logarithmic decrease type having sigmoidal relation with % Radical scavenged.

$EC_{50}$ estimated from the concentration- effect curve for *Tmu* AEBE (Fig. 2- 2b) i.e. $EC_{50}$= 3.63 µg/ml, ranged in accordance to the earlier quantitated value as $EC_{50}$= 1.34 - 4.78 µg/ml having ARP calculated as 20.91-74.62 [13] and $EC_{50}$= 4.0 µg/ml [17].

In comparison to Ascorbic acid ($EC_{50}$= 3.79 µg/ml), *Tmu* AEBE scavenged DPPH$_{fr}$ potentially better. Antiradical Power (ARP) was calculated as 0.275 µg/ml for *Tmu* AEBE and 0.26 for Ascorbic acid.

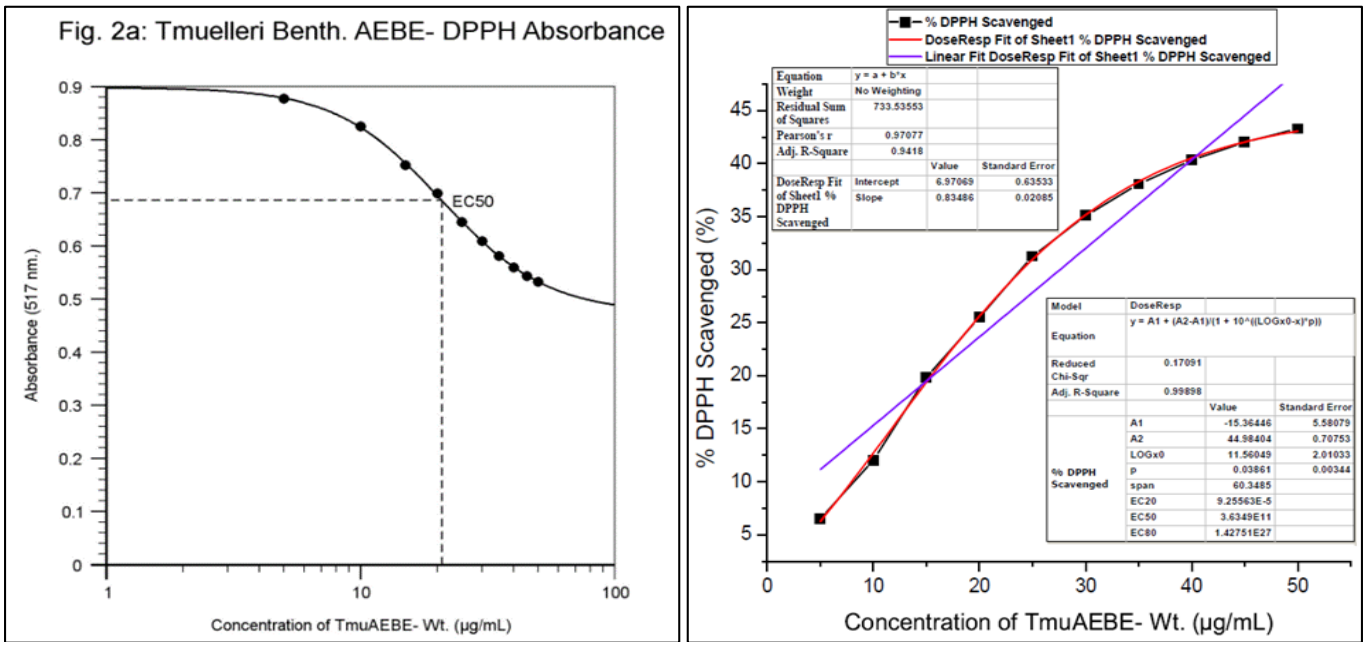


**Fig 2:** DPPH FRSA of *T. muelleri* Benth AEBE

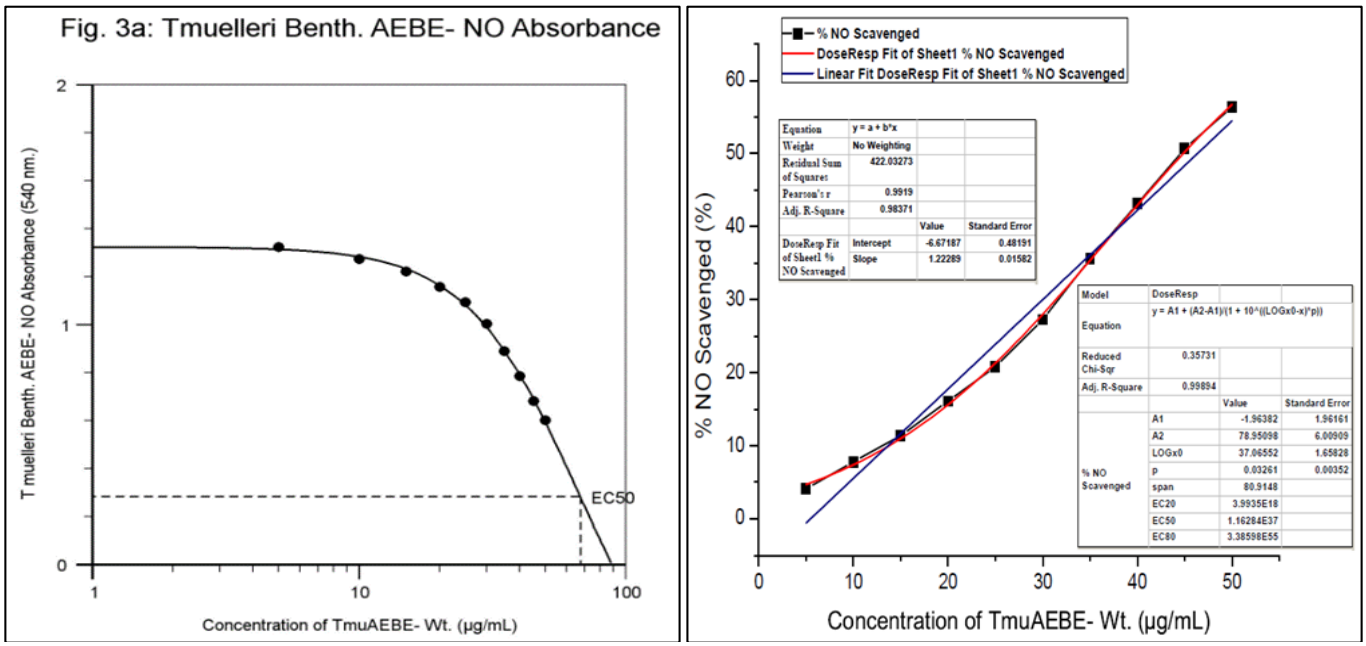


**Fig 3:** NO FRSA of *T. muelleri* Benth AEBE

**Table 1:** *T. muelleri* Benth. FRSA- % Free Radical (DPPH & NO) Scavenged by *Tmu* AEBE**.**

| Sr. no | Wt. (µg/ml) | *DPPH FRSA (% $DPPH_{fr}$ Scavenged) | | #NO FRSA (% $NO_{fr}$ Scavenged) | |
|---|---|---|---|---|---|
| | | Ascorbic acid | *Tmu* AEBE | Ascorbic acid | *Tmu* AEBE |
| 1 | 5 | 2.35 | 6.52 | 4.18 | 4.12 ± 0.328 |
| 2 | 10 | 3.15 | 12.04 | 5.08 | 7.80 ± 0.546 |
| 3 | 15 | 3.95 | 19.86 | 8.84 | 11.43 ± 0.672 |
| 4 | 20 | 5.78 | 25.5 | 11.54 | 16.12 ± 0.142 |
| 5 | 25 | 7.86 | 31.28 | 15.02 | 20.83 ± 0.538 |
| 6 | 30 | 13.06 | 35.1 | 19.56 | 27.28 ± 0.218 |
| 7 | 35 | 18.96 | 38.09 | 24.16 | 35.60 ± 0.140 |
| 8 | 40 | 21.92 | 40.33 | 27.15 | 43.10 ± 0.313 |
| 9 | 45 | 24.2 | 42.03 | 30.74 | 50.66 ± 0.344 |
| 10 | 50 | 27.55 | 43.31 | 31.8 | 56.37 ± 0.568 |
| $EC_{50}$ (µg/ml) | | 33.954 | 20.926 | 35.240 | 68.749 |

**Note:** *Absorbance (ABS) values shown, where recorded after 20 seconds (sec.). This was experimented so as to understand its reaction kinetics (not discussed here).

# % NO FRSA results were expressed as Mean ± S. D. of three replicates (n=3) and analyzed statistically using One way ANNOVA.

**II. *Tmu* AEBE NO FRSA.**

Bioprospecting *Tmu* AEBE for NO FRSA quantitated % NO Radical Scavenged varied from 4.12 % - 56.37% (Table- 1) for experimented concentrations from 5-50 µg/ml. % NO Scavenged for *Tmu* AEBE was found highest [56.37%] at 50 µg/ml. Drop in absorbance, (Fig. 3- 3a) recorded at 540 nm, collated with increased % of NO Scavenged in concentration dependent manner. This decrease in absorbance of formed pink chromophore was an attribute of NO radical abstracted by *Tmu* AEBE and thus used to measure the extent of NO FRS.

$EC_{50}$, estimated by plotting % NO Scavenged against experimented concentrations for *Tmu* AEBE (Fig. 3- 3b) i.e. $EC_{50}$= 1.16 µg/ml was evaluated for the first time and displayed diminished rate than Ascorbic acid [$EC_{50}$= 6.35 µg/ml]. Thus for NO FRSA, *Tmu* AEBE scavenged $NO_{fr}$ much potentially than Ascorbic acid ($P<0.05$).

**Conclusions**

The present experiment affirms potential ability of Bark extract of *T. muelleri* Benth. to scavenge DPPH & NO free radicals, *in-vitro*. *Tmu* AEBE was evaluated for antioxidant activity by two methods– DPPH & NO FRSA and the results were expressed as % Antioxidant Scavenged. $EC_{50}$ value of *Tmu* AEBE for DPPH FRSA [$EC_{50}$= 3. 63 µg/ml] appeared better than its NO FRSA [$EC_{50}$= 1.16 µg/ml], at 50% inhibition. Basis to this, $EC_{50}$ evaluated from % Radical scavenged against concentration curves depicted intense antioxidant activity. The possible reason for this intense sensitivity of *Tmu* AEBE phytochemicals towards DPPH & NO postulated here is that on increasing concentration of *Tmu*AEBE, concentration of constituting phytoconstituents might have raised and thus pronounced this effect. This again clarifies Ayurvedic Pharmacopoeia insight, that high Dose utilization or administration might inflate a Biological activity, here, antioxidant activity of *Tmu* AEBE experimented for DPPH & NO Free Radical Scavenging activity.

**Future Perspective:** As *Tmu* AEBE phytochemicals stoichiometry constitute to antioxidant activity. This analytical phytochemistry, underlying, serves its future perspective and needs to be more Bioprospected.

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