



## INFLUENCE OF SOLVENT EXTRACTION ON TOTAL ANTIOXIDANT CAPACITY OF *VERNONIA AMYGDALINA* AND *LAURUS NOBILIS* LEAVES

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### ABSTRACT

Cells in the body are chronically exposed to oxidants from exogenous and endogenous sources. Nevertheless, antioxidants play important roles in neutralising their effects. The present study investigated the total antioxidant capacity (TAC) of aqueous and methanolic leaf extracts of *Vernonia amygdalina* and *Laurus nobilis* at concentrations of 100, 200 and 300 µg/mL, under in vitro conditions, using the phosphomolybdenum assay. Data were analysed using a significance level of  $p < 0.05$ . Results showed solvent-dependent variations across all tested concentrations. At 100 and 300 µg/mL, *V. amygdalina* methanol extract showed higher values of  $255.70 \pm 10.17$  mg AAE/g DW and  $276.36 \pm 17.18$  mg AAE/g DW compared to the aqueous extract that exhibited  $230.00 \pm 13.83$  mg AAE/g DW at 100 µg/mL and  $246.99 \pm 14.94$  mg AAE/g DW at 300 µg/mL. Likewise, *L. nobilis* extracts recorded substantial increases across all tested concentrations (aqueous extracts:  $222.16 \pm 11.50$  to  $241.18 \pm 10.67$  mg AAE/g DW; methanol extract:  $224.80 \pm 15.37$  to  $255.15 \pm 13.45$  mg AAE/g DW). Both extracts displayed notable antioxidant activity, with the methanolic extract exhibiting higher activity than the aqueous extract. This may be attributed to the enhanced solubility and extraction efficiency of phenolic compounds in methanol. The outcome of this study supports the use of both plants in traditional medicine. However, further studies are required to identify the secondary metabolites responsible for the observed antioxidant activity and their potential application in food.

**Keywords:** Antioxidant, *Laurus nobilis*, Methanolic extract, Traditional medicine, *Vernonia amygdalina*

### INTRODUCTION

Antioxidants are compounds that scavenge reactive oxygen species (ROS) and protect biological systems from oxidative damage (Chandimali, 2025) by either repairing ROS-induced damage or preventing its occurrence (Pham-Huy *et al.*, 2008). This can minimize the risk of developing chronic conditions such as degenerative disorders and cancer (Lobo *et al.*, 2010). Medicinal plants represent one of the oldest forms of treatment in traditional medicine and are widely recognised for their therapeutic benefits (Marrelli, 2021). Their medicinal value is attributed to the presence of phytochemicals, particularly flavonoids and phenolics which possess antioxidant properties capable of scavenging free radicals (Dai & Mumper, 2010). Among these medicinal plants are *Vernonia amygdalina* and *Laurus nobilis*, both of which have been traditionally utilised for various health-related purposes (Edo *et al.*, 2023; Paparella *et al.*, 2022).

*Vernonia amygdalina*, also known as bitter leaf, is a shrub rich in bioactive constituents with therapeutic potential (Adoyi *et al.*, 2025; Anigboro *et al.*, 2014). It is called chusardoki and shawaka in Hausa, onugbu in Igbo, ewuro in Yoruba and origbo in Urhobo language. It possesses antigenotoxic and antioxidant properties (Eraga *et al.*, 2022). It is rich in flavonoids and other secondary metabolites that contribute to its pharmacological potential. Its therapeutic efficacy in ameliorating various diseases has been linked to its oxidant scavenging ability and its capacity to enhance the body's endogenous antioxidant defense system (Oloruntola, 2026). *Laurus nobilis* leaves is commonly referred to as bay leaves (Khodja *et al.*, 2023). Dobrosłavić *et al.* (2022) reported that constituents isolated from *L. nobilis* leaf such as phenols, exhibit significant antioxidant property. Furthermore, several scientific investigations have reported that leaf extracts of *L. nobilis* exhibit anticancer activity (Dias *et al.*, 2014; Loizzo *et al.*, 2007), anticholinergic (Ferreira *et al.*, 2006), anticonvulsant (Sayyah *et al.*, 2002), antidiabetic (Dearlove *et al.*, 2008; Mohammed *et al.*, 2021; Sahin-Basak & Candan, 2013), antifungal (Gumus *et al.*, 2010; Houicher *et al.*, 2016), anti-inflammatory (Mazzio *et al.*, 2016; Matsuda *et al.*, 2000), antimicrobial (Aumeeruddy-Elalfi *et al.*, 2015; Sidika *et al.*, 2013), antioxidant (Brahmi *et al.*, 2015; Muñiz-Márquez *et al.*, 2014; Dias *et al.*, 2014), and neuroprotective (Verdian-Rizi, 2009) activities.

Analysis of TAC in methanolic and aqueous extracts of *V. amygdalina* and *L. nobilis* will improve understanding of the impact of solvents on antioxidant capacity. Therefore, this study will investigate the influence of solvents on the total antioxidant capacity of *V. amygdalina* and *L. nobilis*, thereby advancing knowledge on the effective use of the leaves of both plants in medicine and the food industries.

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### MATERIALS AND METHODS

All chemicals and reagents used in this study were of analytical grade. Methanol was obtained from British Drug House (BDH), Poole, England. Ammonium molybdate and sodium phosphate dihydrate were obtained from JHD (Shantou, Guangdong, China). Sulphuric acid was supplied by Lobachemie (Mumbai, India). Ascorbic acid was purchased from Sigma-Aldrich (Spruce Street, St. Louis, USA).

#### Collection and Identification of Plant Materials

*V. amygdalina* leaves were collected from naturally growing plants within the main campus of the University of Delta, Agbor, Nigeria, following verbal permission obtained from campus security personnel in May 2025, while dried *L. nobilis* leaves were purchased from Baleke Market, Agbor. Selected plant specimens were identified and authenticated by Prof. H. A. Akinnibosun. Voucher numbers were assigned as follows: *Laurus nobilis* Linn (UBH-L555), *Vernonia amygdalina* Delile (UBH-V342). All subsequent analyses were carried out at the Chemical Science Laboratory, University of Delta, Agbor, Nigeria.

### Extract Preparation

Fresh leaves of *V. amygdalina* and dried leaves of *L. nobilis* were separately rinsed with distilled water and air-dried in a well-ventilated environment until constant weight was obtained. Drying durations were 5 and 2 days respectively. The dried leaves were then pulverized into fine powder using an electric blender. Four hundred grams (400 g) of each powdered sample were separately soaked in 1600 mL of methanol and distilled water for 24 hours. The mixtures were filtered through muslin cloth and the filtrates were concentrated using a rotary evaporator at 45°C. The concentrates were further evaporated to dryness on a water bath maintained at 45°C.

The percentage yield (%) of each extract was determined using the equation below:

$$\% \text{ Yield} = \frac{\text{Extract weight (g)}}{\text{Weight of plant sample (g)}} \times 100 \quad (1)$$

(Riyadi et al., 2023)

The dried extracts were stored in labeled specimen containers and kept refrigerated at 4°C until further analysis.

### Preparation of Stock and Working Solutions

Stock solutions were first prepared by dissolving 2 g of each crude extract (dried sample) in a small volume of aqueous Tween 80 solution (5% v/v in distilled water), which acts as a suspending agent to enhance dispersion of the extract. The solution was then made up to a final volume of 100 mL with the same solvent, resulting in a final concentration of 20 mg/mL. The stock solutions were further diluted using the dilution equation ( $C_1V_1 = C_2V_2$ ) to obtain working concentrations of 100, 200 and 300 µg/mL.

### TAC Determination

The method of Prieto et al. (1999) was used. The molybdenum reagent was prepared by mixing 4 mM ammonium molybdate, 28 mM sodium phosphate and 0.6 M sulfuric acid. For each sample, 0.1 mL of the plant extract (at concentrations ranging from 100-300 µg/mL) was mixed with 1.0 mL of the reagent in screw-cap tubes. The tubes were sealed and placed in a heating block at 95°C for exactly 90

minutes. The reaction tubes were allowed to cool to room temperature. During cooling, a distinct colour development was observed in samples containing antioxidants, with the formation of a characteristic green phosphomolybdenum complex. The absorbance of this coloured complex was measured spectrophotometrically at 695 nm. Quantification of total antioxidant capacity was achieved by comparing sample absorbance values against a blank. Ascorbic acid (20-100 µg/mL) was used as standards and the total antioxidant capacity is expressed as ascorbic acid equivalents (AAE)

### Statistical Analysis

Results were expressed as mean ± standard deviation (SD). Data were analysed using one-way analysis of variance (ANOVA) and differences between means were considered significant at 5% confidence level. Post hoc comparisons were determined using the least significant difference (LSD) test.

## RESULTS AND DISCUSSION

### Percentage Yield

For the methanolic extracts, *V. amygdalina* yielded 32 g (8%), while *L. nobilis* yielded 10.4 g (2.6%). For the aqueous extracts, *V. amygdalina* yielded 20.4 g (5.1%), whereas *L. nobilis* yielded 2.4 g (0.6%). From the percentage yield results, the methanolic extract demonstrated a higher yield than the aqueous extract. This could be attributed to differences in solvent polarity. According to Onakurhefe et al. (2026), polarity is a key factor in the extraction process. Since methanol possesses both polar and moderately non-polar properties, it can extract a wider range of phytochemical constituents, whereas water mainly extracts polar constituents (Truong et al., 2019).

### Total Antioxidant Capacity

The findings on the antioxidant properties of *Vernonia amygdalina* and *Laurus nobilis* leaf extracts are presented in Tables 1 and 2. Methanolic leaf extracts of *V. amygdalina* and *L. nobilis* demonstrated higher total antioxidant capacity than their aqueous counterparts across all concentrations, with a concentration-dependent increase observed in both extracts.

**Table 1: Total Antioxidant Capacity of *Vernonia Amygdalina* Leaf Extracts**

Conc. (µg/mL)	Aqueous extract (mg AAE/g DW)	Methanol extract (mg AAE/g DW)
100	230.00 ± 13.83 <sup>c</sup>	255.70 ± 10.17 <sup>a</sup>
200	240.14 ± 16.04 <sup>a</sup>	258.11 ± 15.11 <sup>b</sup>
300	246.99 ± 14.94 <sup>b</sup>	276.36 ± 17.18 <sup>c</sup>

Values are expressed as mean ± standard deviation of triplicate determination (n = 3). Values with different superscript letter differ significantly at (p < 0.05) AAE = Ascorbic acid equivalent.

**Table 2: Total Antioxidant Capacity of *Laurus Nobilis* Leaf Extracts**

Conc. (µg/mL)	Aqueous Extract (mg AAE/g DW)	Methanol Extract (mg AAE/g DW)
100	222.16 ± 11.50 <sup>a</sup>	224.80 ± 15.37 <sup>a</sup>
200	230.69 ± 5.27 <sup>c</sup>	245.81 ± 14.97 <sup>b</sup>
300	241.18 ± 10.67 <sup>b</sup>	255.15 ± 13.45 <sup>c</sup>

Values are expressed as mean ± standard deviation of triplicate determination (n = 3). Values with different superscript letter differ significantly at (p < 0.05) AAE = Ascorbic acid equivalent.

The TAC of *V. amygdalina* increased with concentration (Table 1). The higher TAC observed in the methanolic extracts implies more efficient extraction of antioxidant constituents, highlighting the role of solvent polarity on plant constituent recovery (Eloff, 1998; Ghaffar & Perveen, 2025; Nandhakumar & Indumathi, 2013; Wakeel et al., 2019; Zhang et al., 2019). Furthermore, aqueous extracts exhibited appreciable TAC values, confirming that water-

based preparations of *V. amygdalina* also contain bioactive constituents with free radicals scavenging activity. However, the TAC content of *L. nobilis* (Table 2) also increased with concentration in both extracts, suggesting a dose-dependent enhancement of radical-scavenging activity. It was further observed that higher extract concentrations enhanced the availability of bioactive phytochemicals, particularly phenolic compounds which are known for their

strong antioxidant potentials (Thouri *et al.*, 2017). Antioxidant compounds such as phenolic compounds, act by donating hydrogen atom and transferring of single electron from one molecule to another, thus scavenging ROS and preventing oxidative damage (Losada-Barreiro *et al.*, 2022). The superior performance of methanolic extracts across concentrations suggests that methanol was more efficient in extracting phenolic constituents due to its polarity. This is in conformity with Lee *et al.* (2024), Manye *et al.*, 2023, Onakurhefe *et al.* (2019) and Turay & Kargbo (2025). Aqueous extracts showed reduced antioxidant capacity however, their measurable radical-scavenging potential indicates that water can also extract bioactive constituents but with reduced efficiency. These findings uphold the likely applications of *V. amygdalina* and *L. nobilis* as natural sources of antioxidants for therapeutic study and defense against oxidative stress.

#### Limitation

Detailed phytochemical analysis and total phenolic content determination were not included in the present study. Hence, further studies are recommended to identify the bioactive compounds responsible for the observed antioxidant activity.

#### CONCLUSION

The study demonstrated that both *V. amygdalina* and *L. nobilis* possessed significant TAC, with methanolic extracts showing higher values than aqueous extracts due to enhanced phytochemical solubility in methanol.

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## PLANT IDENTIFICATION LETTER (1)



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**Plant Name:** - *Vernonia amygdalina* Delile

**Family:** Asteraceae

**Common Name:** Bitterleaf

**Voucher Number:** UBH-V342

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**Plant Identification and Voucher Number Issued by:**

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**Plant Name:** - *Laurus nobilis* Linn.

**Family:** Lauraceae

**Common Name:** Bay Laurel, Bay Leaf, True Laurel, Sweet Bay

**Voucher Number:** UBH-L555

**Staff Name:** Dr. Mrs. Patience Onakurhefe

**Plant Identification and Voucher Number Issued by:**

  
29/08/2025

Prof. Akinnibosun Henry Adewale (FLS, MRSB; London, MSWS; USA, MBOSON, MECOSON, MAEIAN, MFBAN; Nigeria)



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