

Polyphenolic Profiles and Antioxidant Potential of Selected Indian *Dioscorea* Species

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Abstract: *Dioscorea* tubers are widely valued in India as both food materials and ethnomedicinal resources, yet direct comparisons of their polyphenolic profile and antioxidant behaviour remain limited. This study compared total phenolic content (TPC), total flavonoid content (TFC), total proanthocyanidin content (TPAC), ferric reducing antioxidant power (FRAP), and nitric oxide (NO) scavenging activity in ten Indian *Dioscorea* species. Methanolic tuber extracts were assessed by Folin–Ciocalteu, aluminium chloride, acidified vanillin, FRAP, and sodium nitroprusside–Griess procedures. Data were reported as mean $\pm$ SD. TPC varied from 2.36 ± 0.05 to 9.68 ± 0.08 mg GAE/g DW, with *D. bulbifera* giving the highest value. TFC ranged between 0.34 ± 0.02 and 0.84 ± 0.03 mg QE/g DW and was greatest in *D. hamiltonii*. TPAC extended from 3.02 ± 0.07 to 6.60 ± 0.06 mg CE/g DW, and *D. bulbifera* again ranked first. FRAP values ranged from 0.067 ± 0.006 to 1.06 ± 0.04 , with the strongest reducing capacity observed in *D. bulbifera* and *D. alata*. NO scavenging IC₅₀ values ranged from 47.31 ± 0.78 to 683.33 ± 10.41 μ g/mL; *D. bulbifera* showed the strongest activity, followed by *D. pubera* and *D. pentaphylla*. Higher TPC and TPAC were associated with lower NO IC₅₀ values and showed moderate positive relationships with FRAP. Marked differences in antioxidant behaviour were evident among the species. Overall, *D. bulbifera* was the most consistently active and should be prioritized for development of nutrition supplement and safety assessment.

Keywords: *Dioscorea* spp; polyphenols; phenolic content; proanthocyanidins antioxidant; activity.

1. Introduction

Aerobic metabolism continuously produces reactive oxygen and nitrogen species that contribute to cell signalling, host defence, and regulation of the redox environment. If their generation surpasses antioxidant protection and cellular repair mechanisms, redox balance can be disrupted, leading to oxidative damage of lipids, proteins, and nucleic acids (Halliwell, 2007; Sies & Jones, 2020). Phenolic constituents of plants can participate in redox reactions by donating electrons or hydrogen atoms, binding transition metals, and influencing oxidation processes. For this reason, foods and medicinal plants remain important sources of redox-active compounds (Apak et al., 2016; Rice-Evans et al., 1996). At the same time, antioxidant assays performed in vitro represent specific chemical reaction mechanisms and should not be considered direct evidence of clinical efficacy (Apak et al., 2016; Gülçin, 2020).

The genus *Dioscorea* L. (Dioscoreaceae) includes food and medicinal yams distributed predominantly in tropical and subtropical regions. Yam tubers supply starch and micronutrients, while their specialized metabolites include phenolic acids, flavonoids, tannin-like compounds, steroidal saponins and allantoin (Baah et al., 2009; Obidiegwu



et al., 2020; Salehi et al., 2019). A few species are eaten locally and cultivated ones are used in India. However, traditional use of plants is seldom backed by an in-depth analysis of the underutilized taxa (Padhan & Panda, 2020). They are subject to genotype, organ, maturity and environment effects in their chemical composition. The post-harvest conditions and handling of the crop during extraction (Adoménienė & Venskutonis, 2022; Lebot et al., 2023; F. Van der Vecht et al., 2019), as well as the processing time, all affect the final product. al., 2023; Obidiegwu et al., 2020). Thus, values from various laboratories or protocols may not be exactly comparable.

Previous research has shown presence of phenolic compounds and antioxidant activity in *D. alata* and the use of wild yam tubers is limited to inbreds (Bhandari & Kawabata, 2004; Sakthidevi & Mohan, 2013), and wider reviews acknowledge *Dioscorea* as a possible source of food associated bioactive compounds (Adoménienė & Venskutonis, 2022; Obidiegwu et al., 2020; Salehi et al., 2019). There is also significant chemical diversity within *Dioscorea* as seen in recent syntheses. The need for a standardized comparative study of species (Lebot et al., 2023; Wang et al.,). However, most of the literature analyzes and considers only one or a few species, and uses varying Solvents or antioxidant endpoints, or reports results on inconsistent mass bases. A concerted effort to assess various Indian species in a side-by-side fashion is therefore required to illuminate the distinction between true and false. Methodological variation is a source of specific patterns.

For the present investigation, 42 *Dioscorea* species reported in India were considered for the present study and investigated by prioritizing the most potent species.

2. Materials and Methods

2.1 Study design and selection of species

Tubers from ten *Dioscorea* species were examined in a comparative laboratory study. Selection was based on a predefined prioritization procedure applied to 42 species documented from India. Each species received a score according to its occurrence in Maharashtra or other parts of India, edible and/or medicinal significance, use of tubers and/or bulbils, distribution, field availability, and cultivation status. The ten highest-ranking species were selected for further studies.

2.2 Chemicals and reagents

Methanol, Folin–Ciocalteu reagent, sodium carbonate, aluminium chloride, sodium nitrite, sodium hydroxide, vanillin, hydrochloric acid, 2,4,6-tripyridyl-s-triazine (TPTZ), ferric chloride hexahydrate and sodium acetate were of analytical or HPLC grade. Gallic acid, quercetin, (+)-catechin and ferrous sulphate were used as calibration standards. Distilled water was used throughout.

2.3 Collection, authentication and processing

Mature tubers at the appropriate physiological stage were collected from natural populations in India during September to December. Identification and authentication were carried out by Dr. Suresh Jagtap, Senior Taxonomist, IRSHA, Pune, using diagnostic morphological features together with comparison against reference herbarium specimens.

Tubers were cleaned to remove adhering soil, washed with water, peeled cut into slices and shade-dried at until constant mass. Dried material was milled in a stainless-steel grinder and passed through a 0.5-mm sieve. Powders were stored in airtight, light-protected polypropylene containers with desiccant at until analysis. Moisture was determined gravimetrically, and all results were expressed per gram of dry sample mass.

2.4 Preparation of methanolic extracts

Extractions were carried out using hot extraction method (using Soxhlet apparatus) for the organic solvents which includes; methanol, ethyl acetate, and hexane. In this method, 15 g of powder was packed into Soxhlet apparatus

and extracted with methanol at 45°C. While aqueous extract was prepared using cold extraction method in which 100 mL of water was added to the 15 g of plant powder and kept in shaker at 30°C for 24 hours at 130 rpm. The cold-water extraction conditions were adapted from Ponnusamy et al. (2011). These extracts were finally concentrated under reduced pressure at 40°C using rotary evaporator (IKA-RV 10, Germany). All dried extracts were stored at 4°C until further use.

2.5 Total phenolic content

TPC was determined by the Folin–Ciocalteu colorimetric method, adapted from Singleton and Rossi (1965). An aliquot of extract (1000 µL; working concentration 50 µg/mL) was mixed with 500 µL diluted Folin–Ciocalteu reagent (2:1) and, immediately thereafter, 2000 µL sodium carbonate (20%, w/v) was added. The mixture was incubated for 2 min at room temperature (25 ± 2 °C) in the dark, and absorbance was read at 650 nm against a reagent blank. Gallic acid (10–50 µg/mL) was used for calibration. Results were calculated from the linear regression equation and expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW). The Folin–Ciocalteu response is an index of total reducing substances and is not chemically specific to phenols (Everette et al., 2010).

2.6 Total flavonoid content

TFC was estimated by an aluminium-chloride colorimetric assay adapted from Chang et al. (2002). Extract at 1 mg/mL (500 µL) was mixed with 100 µL aluminium chloride solution, 100 µL potassium acetate solution, and 2500 µL distilled water, incubated for 30 min at room temperature (25 ± 2 °C), and absorbance was measured at 415 nm. Quercetin (10–50 µg/mL) served as the calibration standard, and results were expressed as mg quercetin equivalents per g dry weight (mg QE/g DW). The selected protocol, wavelength and reaction chemistry must match the flavonoid classes targeted; aluminium-complexation assays provide method-dependent equivalent values rather than an absolute total of all flavonoids (Pękal & Pyrzyńska, 2014).

2.7 Total proanthocyanidin content

TPAC was measured using the acidified vanillin method of Broadhurst and Jones (1978), with modifications. Extract at 1 mg/mL (500 µL) was mixed with 3.0 mL of 4% (w/v) vanillin in methanol and 1.5 mL of concentrated hydrochloric acid. The resulting extract:vanillin:HCl ratio was 0.5:3.0:1.5 (v/v/v). After incubation for 15 min at room temperature (approximately 25°C) in the dark, absorbance was measured at 500 nm against an extract-specific blank prepared without vanillin. (+)-Catechin (20–100 µg/mL) was used for calibration, and the results were expressed as mg catechin equivalents per g dry weight (mg CE/g DW). The reaction time and solvent composition were kept constant, along with temperature. They are an integral component of colour development and should be considered regularly (Broadhurst & Jones, 1978; Makkar et al., 1993).

2.8 Ferric reducing antioxidant power

FRAP was calculated based on Benzie and Strain (1996) with some modifications for plant extracts. While the pH of the FRAP reagent has been optimized for use at pH 3.6, the pH range can be adjusted to 4.5. mM TPTZ in 40 mM HCl, and 20 mM ferric chloride in a 10:1:1 (v/v/v) ratio. Extract at 1 3.0 mL of FRAP reagent was added to mg/mL (100 µL), incubated at 37°C for 4 min, The absorbance was measured at 593 nm. Ferrous sulphate (100–1000 µM) was used for calibration. Data are reported as µmol Fe (II) equivalents/g dry matter. Sample Intrinsic colour and turbidity were corrected by using blanks.

2.9 Nitric oxide scavenging activity

The scavenging activity of NO was determined by using the sodium nitroprusside–Griess reaction as described by Marcocci et al. (1994) adapted to plant extracts. Sodium Different concentrations of each extract were added to nitroprusside (10 mM) and then incubated. at 25°C for 150 min. The samples were then treated with Griess

reagent (1% Sulphanilamide, 2% phosphoric acid and 0.1% naphthylethylenediamine dihydrochloride). The absorbance was taken at 546 nm with quercetin as the positive control. Percentage inhibition was determined by using the formula: $[(A_{\text{control}} - A_{\text{test}})/A_{\text{control}}] \times 100$; A_{control} The absorbance of the control (without extract) is compared to the absorbance in the presence of extract (A_{test}). extract or standard. Activity was expressed as IC_{50} ($\mu\text{g/mL}$), calculated graphically from the inhibition-versus-concentration plot. Lower IC_{50} values indicate stronger NO scavenging activity.

2.10 Quality control and data integrity

Each calibration curve contained at least five non-zero levels and was accepted when $R^2 \geq [0.99]$. Reagent blanks, sample blanks and a positive-control antioxidant ([Trolox/ascorbic acid]) were analysed with each batch. Measurements were performed in three independent assays, and the mean values were reported. Replicate precision was accepted at coefficient of variation $\leq [10]\%$; samples outside this criterion were reinvestigated according to a predefined rule. Results below the validated quantification limit were reported as <LOQ rather than zero. Calibration equations, working ranges, R^2 , limits of detection/quantification and batch-control recoveries should be retained in the study records and summarized in Table 1.

Table 1. Calibration and analytical-performance summary.

Assay	Standard	Range	Regression	R^2	λ (nm)	Reporting unit
TPC	Gallic acid	10–50 $\mu\text{g/mL}$	$y = mx + c$	$[R^2]$	650	mg GAE/g DW
TFC	Quercetin	10–50 $\mu\text{g/mL}$	$y = mx + c$	$[R^2]$	415	mg QE/g DW
TPAC	(+)-Catechin	10–100 $\mu\text{g/mL}$	$y = mx + c$	$[R^2]$	500	mg CE/g DW
FRAP	FeSO_4	100–1000 μM	$y = mx + c$	$[R^2]$	593	$\mu\text{mol Fe(II)/g DW}$
NO scavenging	Quercetin	10–100 $\mu\text{g/mL}$	Graphical IC_{50}	[goodness of fit]	546	IC_{50} ($\mu\text{g/mL}$)

2.11 Statistical analysis

Results were summarized as mean $\pm$ SD, and the supplied mean values were used for species ranking. Replicate-level observations were unavailable, and the dataset did not distinguish biological replicates from technical repeats; therefore, inferential comparisons such as ANOVA with Tukey grouping were not applied. Exploratory Pearson correlation analysis was performed across the mean values of the ten species to investigate relationships of TPC, TFC, and TPAC with FRAP and NO scavenging IC_{50} . These correlations are descriptive at the species level and require confirmation using independent biological replicate data.

3. Results

Total 42 *Dioscorea* species available in India were considered for the analysis (Table 2). On the basis of score given, top 10 species were selected for the further investigation (Table 3).

Table 2. Prioritization matrix for *Dioscorea* species reported from India.

Sr. No.	Plant name	Occurrence	EM	TB	CW	Food	Bulbils	Distribution	Availability	Cultivation	Score
1	<i>D. alata</i> L.	Mh	E, M	T, B	C	3	3	3	3	3	15
2	<i>D. arachidna</i> Prain & Burkill	O-Mh	E	T	w	2	0	2	1	1	6
3	<i>D. belophylla</i> (Prain)	Mh	E, M	T	W	2	0	3	2	2	9
4	<i>D. brandisii</i> Prain & Burkill	O-Mh	E, M	T	W	2	0	1	1	1	5
5	<i>D. bulbifera</i> L.	Mh	E, M	T, B	C, W	3	3	3	3	3	15
6	<i>D. cumingii</i> Prain & Burkill	O-Mh	E, M	T	W, C	3	0	2	1	1	7
7	<i>D. daunea</i> Prain & Burkill	O-Mh	E	T	W	1	0	2	1	1	5
8	<i>D. decipiens</i> Hook.f.	O-Mh	E	T, B	W	2	3	1	1	1	8
9	<i>D. deltoidei</i> Wall. ex Griseb.	O-Mh	E, M	T	W	2	0	2	2	2	8
10	<i>D. esculenta</i> (Lour.)	Mh	E, M	T	C, W	3	0	3	3	3	12
11	<i>D. floribunda</i> M. Martens & Galeotti	O-Mh	E, M	T	W, C	2	0	2	2	2	8
12	<i>D. glabra</i> Roxb.	O-Mh	E	T	W	2	0	3	2	1	8
13	<i>D. hamiltonii</i> Hook.f.	O-Mh	E	T, B	W	2	3	2	2	1	10
14	<i>D. hispida</i> Dennst.	Mh	E, M	T	W, C	2	0	3	2	3	10
15	<i>D. intermedia</i> Thwaites	O-Mh	M	T	W	0	0	2	2	1	5

Sr. No.	Plant name	Occurrence	EM	TB	CW	Food	Bulbils	Distribution	Availability	Cultivation	Score
16	<i>D. japonica</i> Thunb.	O-Mh	E, M	T	W, C	3	0	1	2	2	8
17	<i>D. japonica</i> var. <i>nagarum</i> Prain & Burkill	O-Mh	E, M	T	W, C	3	0	1	2	2	8
18	<i>D. kalkapershadii</i> Prain & Burkill	O-Mh	M	T	W	0	0	2	1	1	4
19	<i>D. kamoonsensis</i> Kunth	O-Mh	E, M	T, B	W	1	2	2	1	2	8
20	<i>D. laurifolia</i> Wall. Ex Hook.f.	O-Mh	E, M	T, B	W	2	1	2	1	1	7
21	<i>D. lepcharum</i> Prain & Burkill	O-Mh	E, M	T	W, C	2	1	3	1	1	8
22	<i>D. listeri</i> Prain & Burkill	O-Mh	E, M	T	W	3	1	2	1	2	9
23	<i>D. longipedicellata</i>	O-Mh	E, M	T	W	2	1	2	1	1	5
24	<i>D. melanophyma</i> Prain & Burkill	O-Mh	E	T	W	2	2	2	1	1	8
25	<i>D. nummularia</i> Lam.	O-Mh	E	T	W, C	2	0	2	2	2	8
26	<i>D. oppositifolia</i> L.	Mh	E, M	T	W	3	2	3	3	2	13
27	<i>D. orbiculate</i> Hook.f.	O-Mh	E	T	C/W	3	0	2	2	2	9
28	<i>D. pentaphylla</i> L.	Mh	E, M	T, B	C/W	2	1	3	2	2	10
29	<i>D. polystachya</i> Turcz.	O-Mh	E, M	T, B	C/W	2	2	1	1	3	9

Sr. No.	Plant name	Occurrence	EM	TB	CW	Food	Bulbils	Distribution	Availability	Cultivation	Score
30	<i>D. prazeri</i> Prain & Burkill	O-Mh	M	T	W	0	0	2	2	0	4
31	<i>D. pubera</i> Blume	O-Mh	M	T, B	C/W	3	2	2	2	1	10
32	<i>D. pyrifolia</i> Kunth	O-Mh	M	T	W	0	0	2	2	2	6
33	<i>D. scortechinii</i> Prain & Burkill	O-Mh	M	T	W	1	0	2	1	1	5
34	<i>D. serpenticola</i> Hoque & P.K.Mukh.	O-Mh	M	T	W	1	0	1	1	0	3
35	<i>D. spicata</i> Roth	O-Mh	E, M	T	W	2	0	2	1	1	6
36	<i>D. tomentosa</i> J.Koenig ex Spreng.	Mh	E, M	T	W	1	0	3	3	1	8
37	<i>D. trinervia</i> Roxb. ex Prain & Burkill	O-Mh	E, M	T, B	W	2	1	2	2	1	8
38	<i>D. vexans</i> Prain & Burkill	Rare	E, M	T	W	2	0	1	2	0	5
39	<i>D. villosa</i> L.	O-Mh	E, M	T	W	1	0	2	1	0	4
40	<i>D. wallichii</i> Hook.f.	Mh	E, M	T, B	W	2	1	3	1	1	8
41	<i>D. wattii</i> Prain & Burkill	O-Mh	E, M	T, B	W, C	2	2	2	1	3	10
42	<i>D. wightii</i> Hook.f.	O-Mh	E, M	T, B	W, C	2	2	2	2	2	10

EM: edible/medicinal importance; **TB:** tuber/bulbil; **CW:** cultivated/wild; **Mh:** Maharashtra; **O-Mh:** outside Maharashtra.

Scoring entries are reproduced as provided by the author

3.1 Species selected

The prioritization matrix was used only to identify species for comparative laboratory evaluation. Based on the predefined scoring criteria and practical availability, the ten selected species listed in Table 3 were taken forward for analysis; the prioritization scores were not included in the statistical evaluation of the chemical endpoints.

Table 3. Selected *Dioscorea* species and specimen information.

No.	Species	Code
1	<i>Dioscorea alata</i> L.	DA
2	<i>Dioscorea bulbifera</i> L.	DB
3	<i>Dioscorea oppositifolia</i> L.	DO
4	<i>Dioscorea esculenta</i> (Lour.) Burkill	DE
5	<i>Dioscorea hamiltonii</i> Hook.f.	DH
6	<i>Dioscorea hispida</i> Dennst.	DHi
7	<i>Dioscorea pentaphylla</i> L.	DP
8	<i>Dioscorea pubera</i> Blume	DPu
9	<i>Dioscorea wattii</i> Prain & Burkill	DWa
10	<i>Dioscorea wightii</i> Hook.f.	DWi

3.2 Polyphenolic indices

Clear interspecies differences were observed in TPC, TFC, and TPAC (Table 4). TPC extended from 2.36 ± 0.05 mg GAE/g DW in *D. hamiltonii* to 9.68 ± 0.08 mg GAE/g DW in *D. bulbifera*, while *D. pentaphylla*, *D. esculenta*, and *D. pubera* showed the next highest numerical values. Variation in TFC was narrower, ranging from 0.34 ± 0.02 mg QE/g DW in *D. hispida* to 0.84 ± 0.03 mg QE/g DW in *D. hamiltonii*. For TPAC, *D. bulbifera* produced the highest value (6.60 ± 0.06 mg CE/g DW), followed numerically by *D. esculenta*, *D. pubera*, and *D. alata*; the lowest value was recorded for *D. hispida* (3.02 ± 0.07 mg CE/g DW) (Table 4).

Table 4. Polyphenolic content of selected *Dioscorea* tubers.

Code	TPC (mg GAE/g DW)	TFC (mg QE/g DW)	TPAC (mg CE/g DW)
DA	4.68 ± 0.07	0.59 ± 0.02	5.89 ± 0.07

DB	9.68 ± 0.08	0.82 ± 0.03	6.60 ± 0.06
DO	2.72 ± 0.05	0.72 ± 0.03	4.17 ± 0.06
DE	6.45 ± 0.11	0.77 ± 0.02	6.15 ± 0.04
DH	2.36 ± 0.05	0.84 ± 0.03	3.44 ± 0.05
DHi	2.40 ± 0.05	0.34 ± 0.02	3.02 ± 0.07
DP	6.51 ± 0.04	0.73 ± 0.03	4.21 ± 0.06
DPu	6.37 ± 0.03	0.82 ± 0.05	6.07 ± 0.03
DWa	2.71 ± 0.05	0.55 ± 0.02	3.15 ± 0.04
DWi	2.40 ± 0.05	0.56 ± 0.03	3.21 ± 0.04

Values are mean ± SD.

3.3 Ferric reducing antioxidant power and nitric oxide scavenging activity

Considerable variation in FRAP was evident among the species (Table 5). The greatest ferric-reducing capacity was measured in *D. bulbifera* (1.06 ± 0.04), with *D. alata* (1.02 ± 0.07) and *D. esculenta* (0.88 ± 0.03) following closely. The lowest FRAP readings were observed for *D. wattii*, *D. wightii*, and *D. pubera*, at 0.067 ± 0.006 , 0.069 ± 0.005 , and 0.089 ± 0.003 , respectively. NO scavenging IC₅₀ values extended from 47.31 ± 0.78 µg/mL for *D.*

bulbifera to 683.33 ± 10.41 $\mu\text{g/mL}$ for *D. hispida*. Accordingly, *D. bulbifera* showed the strongest NO scavenging effect, followed by *D. pubera* (53.93 ± 0.25 $\mu\text{g/mL}$) and *D. pentaphylla* (138.00 ± 3.00 $\mu\text{g/mL}$) (Table 5).

Table 5. Ferric reducing antioxidant power and nitric oxide scavenging activity of selected *Dioscorea* tuber extracts.

Code	FRAP activity*	NO scavenging IC ₅₀ ($\mu\text{g/mL}$)
DA	1.02 ± 0.07	154.33 ± 5.03
DB	1.06 ± 0.04	47.31 ± 0.78
DO	0.79 ± 0.08	400.33 ± 5.03
DE	0.88 ± 0.03	405.33 ± 4.51
DH	0.65 ± 0.02	372.33 ± 5.13
DHi	0.301 ± 0.014	683.33 ± 10.41
DP	0.483 ± 0.013	138.00 ± 3.00
DPu	0.089 ± 0.003	53.93 ± 0.25
DWa	0.067 ± 0.006	669.67 ± 5.03
DWi	0.069 ± 0.005	654.67 ± 4.51

Values are mean $\pm$ SD. FRAP is reported in the unit supplied by the author and must be verified against the calibration method before submission. For NO scavenging, a lower IC₅₀ indicates stronger activity. No significance letters are assigned without replicate-level data.

3.4 Exploratory associations between polyphenolic indices and antioxidant endpoints

When the ten species means were considered, FRAP showed positive relationships with TPC ($r = 0.460$, $p = 0.181$), TFC ($r = 0.388$, $p = 0.268$), and TPAC ($r = 0.596$, $p = 0.069$), although none met the $p < 0.05$ threshold. In contrast, NO scavenging IC₅₀ was negatively related to TPC ($r = -0.787$, $p = 0.007$), TFC ($r = -0.705$, $p = 0.023$), and TPAC ($r = -0.795$, $p = 0.006$), indicating stronger NO scavenging where polyphenolic values were higher. FRAP and NO IC₅₀ also showed a moderate inverse relationship ($r = -0.498$, $p = 0.143$). Because these exploratory correlations use species means only ($n = 10$), they should not be treated as a substitute for inference based on replicate-level observations (Table 6).

Table 6. Exploratory Pearson correlations between polyphenolic indices and antioxidant endpoints across species means.

Comparison	r	p value	Direction	Interpretation
TPC–FRAP	0.460	0.181	Positive	Moderate; not significant
TFC–FRAP	0.388	0.268	Positive	Weak-to-moderate; not significant
TPAC–FRAP	0.596	0.069	Positive	Moderate; not significant

Comparison	r	p value	Direction	Interpretation
TPC–NO IC ₅₀	–0.787	0.007	Inverse	Higher TPC associated with stronger NO scavenging
TFC–NO IC ₅₀	–0.705	0.023	Inverse	Higher TFC associated with stronger NO scavenging
TPAC–NO IC ₅₀	–0.795	0.006	Inverse	Higher TPAC associated with stronger NO scavenging
FRAP–NO IC ₅₀	–0.498	0.143	Inverse	Moderate; not significant

Correlations were calculated from ten species means and are exploratory. Negative correlations with NO IC₅₀ indicate that higher polyphenolic content was associated with stronger scavenging activity. Confirmatory analysis shall be done using independent biological replicates. To replicate data and prespecified multiplicity control.

4. Discussion

This comparative study showed that there is variation among the ten species of *Dioscorea* in India. Significantly altered polyphenolic indices, ferric-reducing power and NO scavenging activity. D. In terms of TPC and TPAC, and the highest FRAP value, *D. bulbifera* exhibited the highest scores while NO had the lowest scores. The most active species in the test panel (IC₅₀) was the most consistent species. The largest population was found with *D. hamiltonii*. shows the highest values of TFC, intermediate values of FRAP and NO scavenging activity, whereas *D. pubera* Despite the low FRAP, combined high TPC and TPAC with strong NO scavenging activity. value.

It may be noted here that these contrasting rank orders demonstrate that the single colorimetric index does not completely account for. antioxidant behavior. The TPC, TFC and TPAC variations among the inter-species are likely to be due to biological factors such as: There are genotypic variations in characteristics of pigmentation, maturation and specialized metabolite in *Dioscorea* germplasm. **One example of these are profiles, which have been employed by both** Adoménienė & Venskutonis (2022) and Lebot et al. (2023) and by Obidiegwu et al. (2020). Bhandari and Kawabata (2004) had observed significant differences in phenolics content, The antioxidant activity among the wild yam tubers is comparable and the earlier study conducted on *D. alata* also corroborates this. A characteristic of all phenolic antioxidants is their presence (Sakthidevi & Mohan, 2013). The data for the present is listed in the table 2 The species that ranked lowest in the list, *D. bulbifera*, had about four times the TPC, and showing the importance of species selection in screening yams for functional-food applications. phytochemical applications.

The correlation between FRAP and TPC, TFC and TPAC were positive, and in the same direction. The electron-donating properties of phenolic compounds, together with the small number of samples (ten) were shown to be a limitation. Species means results in imprecise estimates. The total amount of antioxidants within the sample is determined by the FRAP. The complex of Fe (III) – TPTZ was reduced to Fe (II) – TPTZ (Benzie & Strain, 1996). The In the case of *D. pubera*, its comparatively low FRAP value, despite its high TPC and TPAC, indicates that it is not very antioxidant, or might be quite high in antioxidants while being low in total phenolics. The concentration of phenolics does not entirely reflect the reducing power and it is influenced by the molecular structure and redox. The response may be affected by potential, extraction selectivity and non-phenolic constituents. (Apak et al., 2016; Gülçin, 2020; Rice-Evans et al., 1996).

The inverse relationships of polyphenolic indices to NO IC₅₀ were more pronounced than the corresponding FRAP associations. *D. bulbifera* and *D. pubera*, with high TPC, exhibited the lowest tannin content. *D. bulbifera* and *D. pubera* with high TPC had the lowest tannin content. Low NO IC₅₀ values were observed for TPAC also. In the

sodium nitroprusside–Griess system, scavengers slow the production of nitrite by competing with the reactions of NO with oxygen. (Marcocci et al., 1994). However, this test is chemical and not cellular and the matrix Absorbance may be affected by colour or direct reactions with the Griess reaction. The Colorimetric polyphenol assays also must be interpreted by a qualified person: the Folin–Ciocalteu The reagent reacts with several reducing substances (Everette et al., 2010; Singleton & Rossi (1965), Flavanoids are detected differently by aluminium chloride and Pękal & Pyrzynska, (2014) found that it was unable to detect some subclasses of flavonoids. The vanillin assay is sensitive to condensed-tannin structure and those values are close to those reported by & Pyrzynska (2014), The reaction conditions (Broadhurst & Jones, 1978; Makkar et al., 1993) The applied perspective entails *D. bulbifera*'s priority for compound-level characterization. It ranked first for four of the five endpoints measured because. It is also possible that *D. pubera* may be *D. alata* had high NO scavenging activity and was important because of its high NO scavenging activity. FRAP with intermediate polyphenolic values. Selection for food and nutraceutical feasibility of development should also be taken into account, such as the edibility, antinutritional properties, and processing requirements. The effects, dose, bioavailability, sustainability and safety (Padhan & Panda, 2020; Salehi et al., 2019). Recent reviews on *D. bulbifera* emphasize that it is not only rich in phytochemistry but has documented safety concerns, especially hepatotoxicity, justifying the requirement for safety evaluation prior to The applications span from food and nutraceutical development (Sharma et al.,; Wang et al.,).

Several limitations should be considered when interpreting these findings. The available results do not clarify whether the reported SD values came from independent biological samples or from repeated technical measurements, so valid ANOVA and Tukey comparisons cannot be assigned. Spectrophotometric procedures provide operational or class-equivalent estimates and can be influenced by interference from the sample matrix. FRAP reflects only one electron-transfer mechanism, whereas the NO assay is an in vitro chemical model; therefore, neither method by itself demonstrates biological efficacy. The unit used for FRAP should also be verified against the ferrous calibration calculation. Further studies should use independently collected samples, chromatographic identification of individual metabolites, additional radical-scavenging and cell-based methods, and suitable safety assessment.

5. Conclusion

Using the stated methanolic extraction procedure and spectrophotometric assays, the ten Indian *Dioscorea* species displayed clearly different polyphenolic and antioxidant patterns. *D. bulbifera* emerged as the strongest overall candidate because it had the highest TPC (9.68 ± 0.08 mg GAE/g DW), TPAC (6.60 ± 0.06 mg CE/g DW), and FRAP (1.06 ± 0.04), as well as the lowest NO scavenging IC₅₀ (47.31 ± 0.78 µg/mL). The greatest TFC was found in *D. hamiltonii*, while *D. pubera* and *D. pentaphylla* also demonstrated strong NO scavenging activity. Differences between the FRAP and NO rankings show that antioxidant potential depends on the assay used. The results therefore support further investigation of *D. bulbifera*, together with selected complementary species, through metabolite profiling, biologically relevant validation, and assessment of processing effects, bioavailability, and safety.

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