Phenolic characterization, antioxidant activities, and inhibitory effects of *Physalis angulata* and *Newbouldia laevis* on enzymes linked to erectile dysfunction

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ABSTRACT

This study reports the phenolic composition, antioxidant activity, and capacity of *Physalis angulata* and *Newbouldia laevis* leaves to inhibit enzymes (phosphodiesterase-5′ [PDE-5′], arginase, acetylcholinesterase [AChE], and angiotensin-I converting enzyme [ACE]) linked to erectile dysfunction. High-performance liquid chromatography–diode array detector analysis of the aqueous extracts revealed the presence of phenolic acids (caffeic, ellagic, chlorogenic, and gallic acids) and flavonoids (quercetin, rutin, isoquercitrin, kaempferol, and quercitrin). *N. laevis* exhibited significantly higher inhibitory effects on PDE-5′, arginase, and ACE activities compared to *P. angulata*. There was no significant (\(P < 0.05\)) difference in the AChE inhibitory activities of both extracts. Furthermore, *P. angulata* exhibited lower radical scavenging and chelating abilities compared to *N. laevis*. These findings revealed that *P. angulata* and *N. laevis* leaves are good candidates for the development of functional foods with potentials to improve erectile function.

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Introduction

Today, the use of plant materials as functional foods and nutraceuticals for the treatment of different diseases is on the increase all over the world\(^1\) These could be linked to their phytochemical constituents, which include phenolic acids, flavonoids, and alkaloids. These phytochemicals have several health benefits with little or no side effect compared to synthetic products\(^1,2\). *Physalis angulata* is the most common specie in the genus *Physalis* L. (family; Solanaceae) and highly distributed across regions of the world\(^3,4\). *Newbouldia laevis* (family: Bignoniaceae) is commonly known as a boundary tree which grows mainly in the tropics in Africa\(^5\). The leaves of these plants are usually used in preparations of local dishes, as spices, and also consumed as vegetables in many Nigerian homes. These plants are also used for the management of diabetes, malaria, hepatitis, and inflammation\(^5–8\). However, despite several reports on their biological activities, there is a dearth of information on their effects on biomolecules that mediates erectile dysfunction (ED).

ED is a vascular disease, which occurs in men and involves inability to have penile erection sufficient for sexual satisfaction\(^9\). ED is a public health problem due to its prevalence amongst young and old men with an estimate of 300 million patients by 2025\(^10,11\). Several risk factors have been attributed to ED, including vascular, neurological, hormonal psychological disorders, as well as...
hypertension, diabetes, heart diseases, and oxidative stress.\textsuperscript{[9,12]} Moreover, some enzymes such as phosphodiesterase-5' (PDE-5'), arginase, acetylcholinesterase (AChE), and angiotensin-I converting enzyme (ACE) have been identified to be upregulated in penile corpus cavernosa tissues in ED patients and also used as therapeutic points in the management of this condition.\textsuperscript{[13–15]} Although synthetic inhibitors of PDE-5', arginase, AChE and ACE are effective, however, they pose several harmful effects.\textsuperscript{[1,16]} Hence, the use of herbal remedies, functional foods, and nutraceuticals has been reported to be of good use.\textsuperscript{[1]} In this study, phenolic constituents of \textit{P. angulata} and \textit{N. laevis} were determined as the effects of their aqueous extracts on PDE-5', arginase, AChE and ACE activities.

\textbf{Materials and methods}

\textbf{Sample collection and preparation}

Fresh \textit{P. angulata} and \textit{N. Laevis} leaves were collected from a local farm around Federal University of Technology Akure. The leaves were identified at the Department of Biology, Federal University of Technology Akure, Ondo state. A voucher specimen was deposited in the Herbarium. The leaves were dried at room temperature and pulverized. Twenty gram each was extracted by maceration at room temperature with 100 mL of distilled water. After 48 h, the extracts were filtered, freeze-dried, and kept in sealed vials at \(-4^{\circ}\text{C}\) prior to subsequent analysis.

\textbf{Chemicals and reagents}

PDE-5' (from \textit{Spodoptera frugiperda}) was acquired from Merck Millipore; hippuryl-L-histidyl L-leucine (HHL), \textit{p}-nitrophenylphenylphosphonate, acetylthiocholine iodide, 5,5'-dithiobis-(2-nitrobenzoic acid), AChE (from electric eel, type VI-S), arginase (from bovine liver), and ACE (from rabbit lung) were purchased from Sigma-Aldrich Co. (Steinheim, Germany). All other reagents used were of analytical grade and glass distilled water was used.

\textbf{Quantification of phenolic compounds by HPLC–DAD}

The quantification of phenolic compounds in the extract was carried out according to the method described by Akomolafe et al.\textsuperscript{[17]} using high-performance liquid chromatography coupled with diode array detector (HPLC–DAD). The peaks observed in the chromatogram were confirmed by comparing its retention time with those of reference standards and by DAD spectra (200–600 nm). Calibration curve for gallic acid: $Y = 12683x + 1176.5$ ($r = 0.9993$), chlorogenic acid: $Y = 13074x + 1267.9$ ($r = 0.9991$), caffeic acid: $Y = 11983x + 1371.0$ ($r = 0.9998$), ellagic acid: $Y = 13571x + 1257.4$ ($r = 0.9995$), quercitrin: $Y = 13509x + 1264.7$ ($r = 0.9999$), isoquercitrin: $Y = 12854x + 1186.1$ ($r = 0.9993$), rutin: $Y = 12983x + 1321.6$ ($r = 0.9995$), quercetin: $Y = 13582x + 1196.5$ ($r = 0.9997$), and kaempferol: $Y = 12930x + 1265.8$ ($r = 0.9994$). The analysis was done at ambient temperature and in triplicate. The limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the standard deviation of the responses and the slope using three independent analytical curves. LOD and LOQ were calculated as 3.3 and 10 \(\sigma\)/\(S\), respectively, where \(\sigma\) is the standard deviation of the response and \(S\) is the slope of the calibration curve.

\textbf{PDE-5' inhibition assay}

The PDE-5' assay was determined using the method of Kelly and Butler\textsuperscript{[18]} with slight modification. The method was based on the spectrophotometric measurement of the color intensity of \textit{p}-nitrophenol produced by the catalytic action of PDE-5' at 405 nm. The control experiment was carried out without the addition of extracts. The PDE-5' inhibitory effect of the extracts was calculated and expressed as percentage inhibition.
Arginase inhibition assay

The method of Kaysen and Strecker\textsuperscript{[19]} was followed to determine the effects on arginase activity. This method is based on the spectrophotometric measurement of urea produced from arginine at 450 nm. The control experiment was carried out without the addition of the extracts. The arginase inhibitory capacity of the extracts was calculated and expressed as percentage inhibition.

AChE inhibition assay

The AChE inhibition of the extracts was assessed by a colorimetric method.\textsuperscript{[20]} The AChE activity was measured by JENWAY UV–visible spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C, using acetylthiocholine iodide (100 μL of 0.05 mM aqueous solution) as substrate. The enzyme inhibitory activities of the extracts were expressed as percentage inhibition.

ACE inhibition assay

The effect of the extracts on ACE activity was determined according to the method of Ademiluyi et al.\textsuperscript{[21]} This method was based on the measurement of hippuric acid (Bz-Gly) produced from the substrate (HHL) by the activity of ACE. The hippuric acid produced was redissolved with distilled water and measured at 228 nm.

Measurement of the radicals scavenging capacity of the extracts

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging capacity of \textit{P. angulata} and \textit{N. laevis} extracts was determined using the method of Gyamfi et al.\textsuperscript{[22]} while the ability of the extracts to scavenge hydroxyl radical produced from Fe\textsuperscript{2+}/H\textsubscript{2}O\textsubscript{2}-induced decomposition of deoxyribose was carried out using the method of Halliwell and Gutteridge.\textsuperscript{[23]} The radical scavenging activities of the extracts were subsequently calculated as percentage inhibition. The method of Minotti and Aust\textsuperscript{[24]} and Puntel et al.\textsuperscript{[25]} was used to determine the Fe\textsuperscript{2+} chelating ability of the extracts. The Fe\textsuperscript{2+} chelating ability was subsequently calculated and expressed as percentage Fe chelation ability.

Statistical analysis

All the analyses were carried out in triplicates. The results are expressed as mean ± SD. Statistical comparisons were investigated by one-way ANOVA followed by Duncan’s post-hoc test for multiple comparisons. IC\textsubscript{50} values were calculated using nonlinear regression analysis.

Result and discussion

Phenolic composition

The phenolic components of the extracts derived from \textit{P. angulata} and \textit{N. laevis} were quantified via HPLC–DAD by comparing their retention time and peaks with standard phenolic compounds under the same conditions. The result in Fig. 1A and 1B shows that some phenolic acids (ellagic acid, caffeic acid, chlorogenic acid, and gallic acid) and flavonoids (kaempferol, isoquercitrin, rutin, quercitrin, and quercetin) were detected in both extracts. However, catechin (50.13 ± 0.03 mg/g) and epicatechin (87.59 ± 0.03 mg/g) were present in \textit{N. laevis} but were not detected in \textit{P. angulata}. Furthermore, \textit{N. laevis} extract showed higher levels of gallic acid (49.71 ± 0.03 mg/g), chlorogenic acid (70.86 ± 0.02 mg/g), caffeic acid (146.08 ± 0.02 mg/g), ellagic acid (69.82 ± 0.01 mg/g), rutin (69.82 ± 0.01 mg/g), isoquercitrin (62.76 ± 0.02 mg/g), and kaempferol (49.32 ± 0.03 mg/g) compared to \textit{P. angulata}. Quercitrin (26.37 ± 0.01 mg/g) and quercetin (26.51 mg/g) were significantly lower in \textit{N. laevis} compared to \textit{P. angulata} (42.91 ± 0.02 and 56.74 ± 0.02 mg/g), respectively.
The phenolic composition of *P. angulata* and *N. laevis* extracts revealed that both plants contain appreciable levels of flavonoids and phenolic acids. Several reports have shown that these compounds have multiple biological effects and play a critical role in the treatment and management of degenerative diseases such as diabetes, cancer, cardiovascular, and Alzheimer’s disease.\(^{26-29}\) Moreover, some flavonoid-rich foods have been reported to be effective in the treatment of ED.\(^{30}\) The presence of flavonoids and phenolic acids identified in *P. angulata* and *N. laevis* extracts could be linked to the reported biological activities and also could serve as nutraceuticals with great potentials in improving erectile function.

**PDE-5′ inhibition assay**

The effect of *P. angulata* and *N. laevis* extracts on PDE-5′ activity was investigated at different concentrations (20.27–90.10 µg/mL). *N. laevis* extract had the highest inhibitory effect with IC\(_{50}\) value of 44.25 ± 1.54 µg/mL compared to *P. angulata* extracts (IC\(_{50}\) = 48.98 ± 1.23 µg/mL) (Fig. 2 and Table 2). Sildenafil citrate (PDE-5′ inhibitor) showed higher PDE-5′ inhibitory effect than the extracts (Table 2). PDE-5′ is localized in the corpus cavernosum of penile tissues and plays a major role in penile detumescence, inhibition of platelet, and vasodilation.\(^{31}\) The observed inhibition of PDE-5′ activities by *N. laevis* and *P. angulata* extracts suggests that these plants contain bioactive compounds with promising potentials for the management and treatment of ED, as this could increase cellular concentrations of cyclic-guanosine monophosphate in penile tissues thereby enhancing vasodilation and smooth muscle relaxation.

### Table 1. Phenolic composition of *Physalis angulata* and *Newbouldia laevis* leaf extracts (mg/g).

| S/N | Compounds    | PHY       | NBD       |
|-----|--------------|-----------|-----------|
| 1.  | Gallic acid  | 4.63 ± 0.02\(^b\) | 49.71 ± 0.03\(^a\) |
| 2.  | Catechin     | 5.19 ± 0.01\(^c\) | 70.86 ± 0.02\(^c\) |
| 3.  | Chlorogenic acid | ND | 146.08 ± 0.02\(^c\) |
| 4.  | Caffeic acid | 23.85 ± 0.05\(^d\) | 69.82 ± 0.01\(^b\) |
| 5.  | Ellagic acid | 18.93 ± 0.03\(^b\) | 49.32 ± 0.03\(^a\) |
| 6.  | Epicatechin  | 17.36 ± 0.02\(^a\) | 62.73 ± 0.02\(^ab\) |
| 7.  | Rutin        | 42.91 ± 0.02\(^b\) | 26.51 ± 0.01\(^e\) |
| 8.  | Quercetin    | 34.08 ± 0.01\(^cd\) | 62.73 ± 0.02\(^ab\) |
| 9.  | Isoquercetin | 56.74 ± 0.03\(^b\) | 26.51 ± 0.01\(^e\) |
| 10. | Quercetin    | 18.93 ± 0.03\(^b\) | 49.32 ± 0.03\(^a\) |

Results are expressed as mean ± standard deviations (SD) of three determinations. Averages followed by different letters differ by Tukey test at \(P < 0.05\). PHY: *Physalis angulata*; NBD: *Newbouldia laevis*; ND: not detected.
Arginase inhibitory activity

Figure 3 revealed arginase inhibitory activities of *P. angulata* and *N. laevis* extracts in vitro. Nevertheless, *N. laevis* (IC$_{50}$ = 177.92 ± 4.51 µg/mL) exhibited significantly higher inhibitory activity on arginase compared to *P. angulata* (IC$_{50}$ = 177.92 ± 4.51 µg/mL) (Table 2). Recent investigations have identified arginase as a biomarker for the treatment of sexual dysfunction in men.\(^{[32,33]}\) There is upregulation of arginase activity and low nitric oxide (NO) levels in the corpus cavernosa tissues of ED patients. This condition has been associated with reduced vaso-relaxation of smooth muscles and impaired erection.\(^{[34]}\) However, inhibition of arginase improves NO biosynthesis, smooth muscle relaxation and restores penile erection in ED patients.\(^{[35]}\) This result agrees with the report of Kim et al.\(^{[32]}\) which revealed that *Scutellaria indica* inhibited arginase activities in vitro. Moreover, functional foods have been shown to be effective in the treatment of ED.\(^{[1]}\) Oboh et al.\(^{[35]}\) established the link between the inhibition of arginase activity exhibited by *Moringa oleifera* leaves and their phenolic constituents. Moreover, structure–function relationship has shown that quercitrin and quercetin which were identified in *P. angulata* and *N. laevis* extracts are potent inhibitors of arginase activity and their inhibitory effect was attributed to their hydrophobic interactions with the active site of the enzyme, thereby increasing the pool of arginine and biosynthesis of NO.\(^{[36]}\)

Table 2. IC$_{50}$ values for the inhibition of PDE-5', arginase, AChE, and ACE activities, as well as DPPH, OH radical scavenging, and Fe$^{2+}$ chelating capacities of *Physalis angulata* and *Newbouldia laevis* leaf extracts.

| Parameter | *P. angulata* | *N. laevis* | Positive control |
|-----------|--------------|--------------|-----------------|
| PDE-5' (µg/mL) | 48.98 ± 1.23$^c$ | 44.25 ± 1.54$^b$ | 2.78 ± 0.11$^d$ |
| Arginase (µg/mL) | 177.92 ± 4.51$^a$ | 134.98 ± 3.23$^b$ | 0.75 ± 0.20$^e$ |
| AChE (mg/mL) | 0.60 ± 0.04$^b$ | 0.53 ± 0.05$^b$ | 2.44 ± 0.19$^a$ |
| ACE (µg/mL) | 269.69 ± 2.30$^b$ | 130.00 ± 3.11$^c$ | 0.22 ± 0.03$^d$ |
| DPPH (mg/mL) | 3.62 ± 0.25$^a$ | 2.66 ± 0.10$^b$ | - |
| OH (mg/mL) | 0.52 ± 0.04$^a$ | 0.42 ± 0.07$^a$ | - |
| Fe$^{2+}$-chelation (mg/mL) | 1.65 ± 0.11$^d$ | 0.82 ± 0.05$^c$ | - |

Values represent means of triplicate readings. Values along the same rows with different superscripts are significantly different at $P < 0.05$. Positive control; sildenafil citrate, *N*-hydroxy-L-arginine (NOHA), prostigmine (µg/mL)*, and lisinopril were used as PDE-5', arginase, AChE, and ACE inhibitors, respectively. --: Not determined.
AChE inhibitory activity

*P. angulata* and *N. laevis* extracts showed inhibitory effects on AChE activity in a dose-dependent manner as illustrated in Fig. 4. Moreover, the IC<sub>50</sub> values in Table 2 revealed that there was no significant difference (*P* > 0.05) between the inhibitory effects of *P. angulata* (0.60 ± 0.04 mg/mL) and *N. laevis* (0.53 ± 0.05 mg/mL) extracts on AChE activity. The AChE inhibitory activity of the extracts was significantly lower than prostigmine (positive control) as shown in Table 2. Furthermore, the release of NO in the corpus cavernosum tissue has been shown to be dependent on cholinergic nerves.<sup>[37]</sup> Previous research have demonstrated that cholinergic nerves are present in the penile tissues and are capable of releasing acetylcholine, which binds to muscarinic receptors and stimulate NO synthesis via activation of endothelial NO synthase.<sup>[13]</sup> In Fig. 3, we observed that *P. angulata* and *N. laevis* extracts reduced AChE activity which can increase the pool of acetylcholine in penile tissues. Our findings revealed that the use of *P. angulata* and *N.*
*laevis* may be a novel approach to alleviate psychogenic ED as cerebral impulse has been reported to initiate the release of NO and acetylcholine.\[^{38}\]^  

### ACE activity

Angiotensin II (Ang II) is a peptide which stimulates smooth muscle contraction and contributes to the development of ED.\[^{39,40}\]^ This peptide is formed in the renin–angiotensin systemic pathway in a reaction catalyzed by ACE.\[^{41}\]^ High levels of Ang II have been observed in the corpus cavernosum of ED patients.\[^{42}\]^ Previous reports have also shown that drugs that can reduce Ang II levels via inhibition of ACE activity could improve erectile function.\[^{13,43}\]^ Figure 5 reveals the effect of the extracts on ACE activity. *P. angulata* and *N. laevis* reduced ACE activity. The IC\(_{50}\) in Table 2 revealed that *P. angulata* extract (269.69 ± 2.30 µg/mL) had significantly (*P* < 0.05) lower inhibitory effect on ACE activity compared to *N. laevis* (130.00 ± 3.11 µg/mL). However, lisinopril (IC\(_{50}\) = 0.22 ± 0.03 µg/mL) exhibited significantly (*P* < 0.05) higher inhibitory activity than the extracts (Table 2). This result suggests that the extracts may contain potent ACE inhibitors that are capable of improving erectile response as well as sexual satisfaction in ED patients.

### Antioxidant activity

Evidence from previous studies has revealed that oxidative stress mediated through free radicals may impair cavernosal function in ED patients.\[^{44}\]^ Free radicals especially superoxide react with NO to form radicals such as peroxynitrite thereby impairing transmission of NO and smooth muscle relaxation which could lead to penile flaccidity.\[^{45–47}\]^ However, antioxidants have been reported to play an important role in erectile function due to their ability to mop up free radicals and chelate transition metals.\[^{48,49}\]^ In this study, the capacities of *P. angulata* and *N. laevis* to scavenge free radicals and chelate Fe\(^{2+}\) were determined. Interestingly, both extracts scavenged DPPH and OH radicals and were able to chelate Fe\(^{2+}\) in a dose-dependent manner. The IC\(_{50}\) values in Table 2 showed that the highest DPPH radical scavenging activity was exhibited by *N. laevis* (2.66 ± 0.10 mg/mL). *P angulata* showed an IC\(_{50}\) of 3.62 ± 0.25 mg/mL. Both extracts also scavenged OH radicals and prevented the degradation of deoxyribose. The result obtained in Table 2 revealed that there was no significant (*P* > 0.05) difference between the OH radical scavenging activity of *P angulata* (0.52 ± 0.04 mg/mL) and *N. laevis* (0.42 ± 0.07 mg/mL), while *P. angulata* extract exhibited a

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**Figure 5.** Inhibitory effect of *P. angulata* and *N. laevis* on angiotensin-I-converting enzyme activity. PHY: *Physalis angulata*; NBD: *Newbouldia laevis*. 
lower Fe chelating ability (Table 2). The observed OH radical scavenging and metal chelating capacities of the extracts may prevent oxidative stress and protect the penile tissues against oxidative damage induced by-products of lipid peroxidation. Fe has been implicated as a catalyst and mediator in the reaction involving peroxyl radicals and lipid molecules which triggers a chain reaction leading to lipid peroxidation.[44]

Conclusion

This study revealed that *P. angulata* and *N. laevis* leaves contain appreciable levels of phenolic acids and flavonoids particularly chlorogenic acid, caffeic acid, epicatechin, ellagic acid, rutin, and quercetin. The aqueous extracts showed inhibitory effects on PDE-5′, arginase, AChE, and ACE activities and exhibited radical scavenging and metal chelating activities which could be linked to their phenolic constituents. The phenolic composition, enzyme inhibitory properties, and antioxidant activities of these plants suggest their potentials as functional foods and/or nutraceuticals for the management of ED. However, aqueous extract of *N. laevis* exhibited higher potentials to improve erectile function compared to *P. angulata*.

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