PREPARATION OF PHYTOL-LOADED NANOEMULSION AND SCREENING FOR ANTIOXIDANT CAPACITY: DATA-IN-BRIEF

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ABSTRACT

Prepared phytol-loaded nanoemulsion (PNE) and nano-free (PFNE) phytol (PYT) (2-16 μM) were tested for antioxidant potentials by adapting six in vitro, one ex vivo and one in vivo test methods. As standard torlox was taken in in vitro and ex vivo studies, while hydrogen peroxide (H₂O₂) was considered as a stressor in in vivo test performed in one non-mutant, three single and two double mutants Saccharomyces cerevisiae strains. This record is pointed out to expose the analyzed data, predicting antioxidant potentials of PYT.

KEYWORDS: Antioxidant; Radicals; Nanoemulsion; Phytol; Saccharomyces cerevisiae.
GRAPHICAL ABSTRACT

Abbreviation: ABTS: 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); Cat: catalase (mutant); Cat1Δ: cytoplasmic catalase; DPPH: 1,1-diphenyl-2-picrylhydrazyl; NO: nitric oxide; OH: hydroxyl radical; PdI: diameter index; PFNE: phytol without nano-emulsion; PNE: phytol in nanoemulsion; PYT: phytol; RP: reduction potential; Sod: superoxide dismutase (mutant); SOD-WT: superoxide dismutase-wild type; Sod1Δ: cytoplasmic superoxide dismutase; Sod2Δ: mitochondrial superoxide dismutase; TBARS: thiobarbituric acid; TRO: trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); VNE: vehicle for nanoemulsion; ZP: zeta potential.

Specifications

| Subject area | Biotechnology in natural products |
|--------------|-----------------------------------|
| More specific subject area | Drug discovery and development |
| Type of data | Tables and Figures |
| How data was acquired | Experimental data processed in GraphPadPrism (version: 6.0) |
| Data format | Analyzed and plotted |
| Experimental factors | Antioxidant capacity was calculated as a percentage (%) inhibition of activity |
| Experimental features | In *in vitro* and *ex vivo* studies after application of sample, absorbance measurement was carried out, whereas the *in vivo*, *S. cerevisiae* test followed to the measurement of inhibition of zones |
| Data source location | Laboratory of Experimental Neurochemistry, Nuclear of Pharmaceutical Technology, Federal University of Piauí, Teresina (Piauí), Brazil. |
| Data accessibility | Main article: doi: 10.3823/1941 [International Archives of Medicine 2016; 9(70): 1-15] |

1. Value of the data

Herewith we offer to the readers’ antioxidant capabilities of PFNE and PNE compared to the standard drug, trolox (TRO) (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

We offer to the readers percentage inhibition of free radicals namely – DPPH*, ABTS**, *OH, NO*, TBARS along with reduction potential and hemolysis inhibition profile.

We also offer oxidant/antioxidant profile in *S. cerevisiae* strains.
Values are presented as percentage activity in Tables; whereas percentage inhibition and/or simply inhibition activities in Figures.

2. Data

Table 1 indicates the diameter (%), diameter index (PdI) and zeta potentials (ZP) of PYT in nano-vehicle and PYT-loaded nanoemulsion. Percentage antioxidant activity differences in *in vitro* and *ex vivo* studies were shown in Table 2 and 3. Antioxidant defense in percentage is given in Table 4.

Antioxidant activity in *in vitro* and *ex vivo* studies is given in Table 1, while Table 2 and 3 suggests the antioxidative defenses in *S. cerevisiae* strains.

**Figure 1** shows antioxidant activity in *in vitro* and *ex vivo* models, whereas **Figure 2** and 3 suggest inhibition zones of *S. cerevisiae* with and without nullified STR (H₂O₂).

Table 1. Droplets, diameter index and zeta potential of loaded PYT formulations

| Formulation code | Diameter (% increased) | PdI (% increased) | ZP (mV) ± SD |
|------------------|------------------------|-------------------|--------------|
| VNE             | -                      | -                 | -108 ± 3.01  |
| PNE             | 88.56                  | 107.57            | -77.8 ± 2.83 |

VNE: vehicle for nanoemulsion; PNE: phytol loaded nanoemulsion; PdI: diameter index; ZP: zeta potential

Table 2. Percentage antioxidant potential differences between PFNE and PNE

| Samples (µM) | DPPH (% radical scavence) | ABTS (% radical scavence) | OH (% radical scavence) | NO (% radical scavence) |
|--------------|---------------------------|---------------------------|-------------------------|-------------------------|
|              | PFNE| PNE| PFNE| PNE| PFNE| PNE| PFNE| PNE| PFNE| PNE| PFNE| PNE|
| 16           | -   | 15.33 | -   | 8.41 | -   | 23.55 | -   | 11.48 |
| 8            | 27.05 | -   | 2.01 | -   | -   | 0.70  | -   | 13.11 |
| 4            | 45.69 | -   | 9.61 | -   | 11.67 | -   | -   | 45.41 |
| 2            | 59.36 | -   | 2.10 | 36.79 | -   | 43.90 | -   | -   |
|              | TBARS (% inhibition of lipid peroxidation) | RP (% inhibition oxidation) | HL (% inhibition hemolysis) |
|              | PFNE| PNE| PFNE| PNE| PFNE| PNE| PFNE| PNE|
| 16           | -   | 11.41 | -   | 36.19 | 34.30 | -   |
| 8            | 0.00 | 0.00 | -   | 80.43 | -   | 15.23 |
| 4            | 0.00 | 0.00 | -   | 100.10 | 18.28 | -   |
| 2            | 128.04 | -   | 87.20 | -   | 28.09 | -   |

DPPH: 1,1-diphenyl-2-picrylhydrazyl; ABTS: 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); OH: hydroxyl radical; NO: nitric oxide; TBARS: thio barbituric acid RP: reduction potential; PFNE: phytol without nanoemulsion; Values are percentage (inhibition) in comparison to each other.
Table 3. Percentage antioxidant potential differences between TRO and PFNE/PNE

| Samples (µM) | DPPH (% radical scavenge) | ABTS (% radical scavenge) | OH (% radical scavenge) | NO (% radical scavenge) |
|--------------|---------------------------|---------------------------|------------------------|------------------------|
|              | PFNE | PNE | PFNE | PNE | PFNE | PNE | PFNE | PNE |
| 16           | 51.84 | 31.67 | 11.30 | 2.67 | 36.03 | 10.10 | 27.98 | 14.80 |
| 8            | 54.48 | 96.27 | 23.86 | 26.35 | 35.68 | 34.74 | 46.85 | 29.83 |
| 4            | 14.25 | 66.46 | 24.43 | 13.52 | 35.77 | 51.62 | 69.88 | 16.83 |
| 2            | -    | 34.27 | -    | -    | 4.71  | 43.24 | 184.51 | 309.41 |

Table 4. Antioxidative defense of PFNE/PNE in S. cerevisiae strains stressed with H₂O

| Samples (µM) | SOD-WT | Sod1Δ | Sod2Δ |
|--------------|--------|-------|-------|
|              | PFNE   | PNE   | PFNE  | PNE  | PFNE  | PNE  | PFNE  | PNE  |
| 16           | 49.38  | 51.85 | 45.92 | 74.49 | 67.71 | 67.71 | 65.63 | 66.67 |
| 8            | 53.09  | 65.43 | 71.43 | 66.33 | 65.63 | 66.67 | 68.75 | 65.63 |
| 4            | 53.09  | 60.49 | 77.55 | 69.39 | 68.75 | 65.63 | 75.25 | 69.39 |
| 2            | 59.26  | 72.84 | 78.57 | 75.51 | 82.29 | 77.08 | -     | -     |

| Samples (µM) | Cat1Δ | Sod1Δ | Sod2Δ | Sod1Δ | Cat1Δ |
|--------------|-------|-------|-------|-------|-------|
|              | PFNE  | PNE   | PFNE  | PNE  | PFNE  | PNE  | PFNE  | PNE  |
| 16           | 72.92 | 71.88 | 66.34 | 67.33 | 31.37 | 39.22 | 39.22 | 39.22 |
| 8            | 79.17 | 75.00 | 72.28 | 64.36 | 39.22 | 33.33 | 39.22 | 33.33 |
| 4            | 68.75 | 65.63 | 64.36 | 75.25 | 45.10 | 39.22 | -     | -     |
| 2            | 84.38 | 79.17 | 65.35 | 76.24 | 56.86 | 52.94 | -     | -     |

TRO: trolox (6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid); Values are percentage (inhibition) in comparison to TRO.

SOD-WT: superoxide dismutase-wild type; Sod: superoxide dismutase (mutant); Cat: catalase (mutant); Values are percentage antioxidative defense in comparison to TRO.
Figure 1. Antioxidant activity in in vitro and ex vivo tests.
Figure 2. Inhibition zones (mm) in *S. cerevisiae* strains with nullified strains.

\( p < 0.05 \) compared to NC (0.05% tween 80 in 0.9% NaCl) (one-way ANOVA followed by Bonferroni’s test within 95% confidence interval); values are the mean ± SEM (n=4); Cat1Δ: cytoplasmic catalase; Sod1Δ: cytoplasmic superoxide dismutase; Sod2Δ: mitochondrial superoxide dismutase.
Figure 3. Inhibition zones (mm) in *S. cerevisiae* strains with stressor (H₂O).

\(^{a}p < 0.05\) compared to NC (0.05% tween 80 in 0.9% NaCl); \(^{b}p < 0.05\) compared to STR (H₂O₂); \(^{c}p < 0.05\) compared to PFNE 16 µM; \(^{d}p < 0.05\) compared to PFNE 8 µM; \(^{e}p < 0.05\) compared to PFNE 4 µM (one-way ANOVA followed by Bonferoni’s test within 95% confidence interval) (n=4).

3. Experimental Design, Materials and Methods

3.1. Reagents and chemicals

TRO, PYT and all the necessary reagents and chemicals were collected from Sigma-Aldrich, St. Louis, MO; USA.

3.2. Preparation of PYT-loaded nanoemulsion, analysis of size distribution and measurement of zeta potential

PNE was prepared by emulsion phase inversion method.\(^{[1]}\) The mean droplet diameter was calculated by using the cumulative method of analysis, according to the intensity of the light scattered (DLS) in a particle size analyzer (Brookhaven Instruments, USA), at 659 nm wavelength, 90° detection angle, and at 25°C. The correlation worked in parallel mode, and
data analyzed by using Zeta Plus® Particle Sizing version 3.95 software. ZP measurement was performed in the same equipment, applying field strength at about 5.9 V/cm.

3.3. **Experimental animals:** For hemolysis inhibition test, Wister albino rats (*Rattus norvegicus*) of 180-220 g body weight (8 weeks old) were used.

3.4. **Preparation of test samples:** PFNE/PNE/TRO was emulsified in 0.05% tween 80 dissolved in 0.9% NaCl solution to attain final concentration ranges from 2 - 16 µM. For *S. cerevisiae* assay, PYT (PFNE/PNE) was adjusted with the same dose as above in the same vehicle (sterile). H₂O₂ at a dose of 10 mM was taken as stressor (STR).

3.5. **In vitro antioxidant activity assays**

3.5.1. **DPPH** *(1,1-diphenyl-2-picrylhydrazyl) scavenging activity*  
This test was done by using a slightly modified method as described earlier by Manzocco et al.[2] Briefly, 0.3 ml samples with concentrations from 2 - 16 µM were added to 2.7 ml ethanolic solution of DPPH (0.5 mM). After 30 min, the absorbance was measured by a spectrophotometer at 517 nm. A positive control group was performed with similar concentrations of TRO and as a negative control (NC), only 0.3 ml vehicle was added to the DPPH solution. The blank contains no sample. The DPPH radical scavenging potential was calculated using the equation as given below

\[
\text{% inhibition of DPPH radical} = \left(\frac{A_{br} - A_{ar}}{A_{br}}\right) \times 100
\]

where, \(A_{br}\) is the absorbance of DPPH free radicals before reaction and \(A_{ar}\) is the absorbance of DPPH free radicals after reaction.

3.5.2. **ABTS***(2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) radical cation decolorization assay**  
This test was done with the slight modification of the method reported earlier by Seeram et al.[3] To perform this test, ABTS cationic radicals (ABTS*) were produced by adding solid manganese dioxide (80 mg) to a 5 mM aqueous stock solution of ABTS (in 75 mM Na⁺/K⁺ buffer of pH 7.0). Then 2.8 ml sample was added to 0.2 ml of ABTS* solution. The absorbance was read at 750 nm after 5 min of sample application. The percentage of scavenging capacity was calculated as follows

\[
\text{% scavenged ABTS}^* = \left(\frac{A_{br} - A_{ar}}{A_{br}}\right) \times 100
\]

where, \(A_{br}\) is the absorbance of ABTS* free radicals before reaction and \(A_{ar}\) is the absorbance of ABTS* free radicals after reaction with test samples.
3.5.3. **Hydroxyl radical (•OH) scavenging activity**

The ability of the test sample to scavenge hydrogen peroxide (H$_2$O$_2$) can be estimated according to the method of Ruch et al.$^{[4]}$ Briefly, a solution of H$_2$O$_2$ (40 mM) was prepared in phosphate buffer (50 mM, pH 7.4). The concentration of H$_2$O$_2$ was determined by spectrometrically. Test samples and standard (2 - 16 µM) were added to H$_2$O$_2$ and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without H$_2$O$_2$. The percentage of H$_2$O$_2$ scavenging was calculated as follows:

\[
\% \text{ scavenged } H_2O_2 = \left( \frac{A_0 - A}{A_0} \right) \times 100
\]

where, $A_0$ = absorbance of control and $A$ = absorbance of test.

3.5.4. **Nitric oxide (NO*) scavenging activity**

In this test, the reaction mixture contains 0.375 ml of PNE/FNE/TRO, 1.5 ml of sodium nitroprusside (10 mM) and 0.375 ml phosphate buffer saline (pH 7.4). Then the absorbance ($A_{br}$) was taken at 546 nm. After incubating the reaction mixture at 37 °C for 1 hr, 1 ml solution was mixed with 1 ml of Griess reagent [(1.0 ml sulfanilic acid reagent (0.33% in 20% glacial acetic acid at room temperature for 5 min with 2 ml of naphthylethylenediamine dichloride (0.1% w/v)]. Then the reaction mixture was kept at room temperature for 30 minutes and the final absorbance ($A_{ar}$) was measured at the same wavelength. For NC, only 0.375 ml vehicle was used.$^{[5]}$ The amount of nitric oxide radical (NO*) inhibition was calculated by the following equation:

\[
\% \text{ inhibition of NO radical} = \left( \frac{A_{br} - A_{ar}}{A_{br}} \right) \times 100
\]

where, $A_{br}$ is the absorbance of NO* free radicals before reaction and $A_{ar}$ is the absorbance of NO* free radicals after reaction with the Griess reagent.

3.5.5. **Evaluation of the antioxidant potential against the formation of TBARS**

The TBARS (thiobarbituric acid radicals) assay was adopted to measure the quantity of lipid peroxidation capacity. Slightly modified method in briefly, 0.1 ml sample was added to 1 ml homogenized egg yolk (1% w/v) in 20 mM phosphate buffer (pH 7.4). Then lipid oxidation was induced by the addition of 0.1 ml of 2,2'-azobis(2-methylpropionamide) dihydrochloride solution (AAPH; 0.12 M). The reaction mixture was incubated at 37 °C for 15 minutes. After cooling, 0.5 ml of the sample was centrifuged with 0.5 ml of trichloroacetic acid (15%) at 1,200 × g for 10 min. An aliquot of 0.5 ml of the supernatant was mixed with 0.5 ml TBA (0.67%) and heated at 95 °C for 30 min. After cooling, absorbance was measured using a spectrophotometer at 532 nm. The levels of lipid peroxides were expressed as nM of...
TBARS/mg egg yolk using an extinction coefficient of $1.56 \times 10^5$ ml/cm. Finally, the results were expressed as the percentage of inhibition of lipid peroxidation.$^{[6]}$

3.5.6. **Reduction potential test (RP)**

The RP test was conducted by the modified method as described by Oyaizu.$^{[7]}$ For this, a 0.2 ml of sample was added to 0.5 ml of 0.2 M phosphate buffer (pH 6.6) and 0.5 ml of $K_3Fe(CN)_6$ (1% w/v) and the reaction mixture was heated at 50 °C for 20 min. Then 0.5 ml of trichloroacetic acid (10% w/v) was added with constant shaking, followed by the addition of 1.175 ml distilled water and 0.125 ml of $FeCl_3$ (0.1%, w/v) after 5 minutes. Sample absorbance was taken at 700 nm against blank sample. To NC, only 0.2 ml of the vehicle was added. The reduced power capacity activity is calculated as follows:

Reducing potential (%) = $\frac{(A_{ts} - A_{bs})}{A_{ts}} \times 100$

where, $A_{ts}$ is the absorbance of test sample and $A_{bs}$ is the absorbance of blank sample.

3.6. **Ex vivo antioxidant activity assay**

3.6.1. **Inhibition of $H_2O_2$ induced hemolysis in rat erythrocytes**

In this test, blood was collected from the retro-orbital plexus from anesthetized (sodium pentobarbital 35 mg/kg; intra-peritoneal) Wistar rat and the erythrocytes (RBC) were prepared for 10% suspension in the phosphate buffer saline (PBS pH 7.4). Then to 0.5 ml 10% RBC suspension 0.15 ml $H_2O_2$ (200 mM in PBS, pH 7.4) and 0.2 ml of the sample solution was added. The reaction mixture was then incubated at 37 °C for 30 minutes and centrifuged (2,500 g, 3 min) immediately, followed by withdrawing of 0.2 ml supernatant to mix with 2.8 ml PBS. Then the absorbance was taken at 475 nm.$^{[8]}$ For NC, 0.2 ml of the vehicle was added. The percentage of haemolysis was calculated taking into account the 100% hemolysis induced by the $H_2O_2$ (blank), which used the following formula:

Inhibition of hemolysis (%) = $\frac{(Abs_{blank} - Abs_{sample}) \times 100}{Abs_{blank}}$

where, $Abs_{blank}$ is the control absorbance with 100% hemolysis induced by $H_2O_2$ and reactive $Abs_{sample}$ is the absorbance of aliquot containing concentration of the sample under study.

3.7. **In vivo antioxidant activity assay**

3.7.1. **Oxidant/antioxidant capacity in S. cerevisiae cells**

This test was performed according to the aerobic metabolism pathway earlier described by Fragoso et al.$^{[9]}$ Then, previously sub-cultured strains were linearly swabbed to the sterile YEPD media (0.5% yeast extract, 2% peptone, 2% dextrose and 2% bacteriological agar). Afterwards, 0.01 ml of test samples/controls (specified concentrations) applied on sterile...
paper disks and was treated accordingly, such as – for alone and co-treatment groups the test samples (PFNE/PNE) and the samples plus stressor were added, respectively. The NC and STR groups were treated with sterile vehicle (0.05% tween 80 dissolved in 0.9% NaCl) and H₂O₂, respectively. Treatments were done immediately after the swabbing of organisms in petri-dishes. The dishes were then inverted (180° angle) and kept in an incubator maintaining their temperature at 35 ± 1 °C for 72 h, followed by the measurement of the inhibition zones in millimeters (mm) with a range from 0 mm (full grown) to 40 mm (no growth), these values being the size of the petri-dishes. All of the treatments were performed in duplicate.

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CONFLICT OF INTEREST
None declared.

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