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## Supplementary data to:

# *HYGROPHORUS EBURNEUS,* EDIBLE MUSHROOM, A PROMISING NATURAL BIOACTIVE AGENT

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**Supplementary Table 1:** Observed absorbances of effect of HEAE (in concentration range from 125 to 1000 µg/mL) in acetylcholinesterase inhibition (Raw data concerning **Figure 1**)

| | | Absorbance (412 nm) | | |
|---|---|---|---|---|
| | Concentration (µg/mL) | n1 | n2 | n3 |
| Control | *0* | 2.688 | 2.687 | 2.691 |
| *Hygrophorus eburneus* | *1000* | 1.521 | 1.443 | 1.358 |
| | *500* | 1.774 | 1.703 | 1.841 |
| | *250* | 2.054 | 1.999 | 2.105 |
| | *125* | 2.334 | 2.367 | 2.301 |
| Galanthamine | *1000* | 0.143 | 0.107 | 0.376 |
| | *500* | 0.247 | 0.311 | 0.483 |
| | *250* | 0.654 | 0.606 | 0.811 |
| | *125* | 0.948 | 0.778 | 0.846 |

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**Supplementary Table 2:** Observed absorbances of effects of HEAE (in concentration range from 1 to 500 µg/mL) on viability of HCT-116 and MDA-MB-231 cells (Raw data concerning **Figure 2**)

| Time of treatment | Concentration (µg/mL) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | control *0* | *1* | *10* | *50* | *100* | *250* | *500* | control *0* | *1* | *10* | *50* | *100* | *250* | *500* |
| **24 h** | HCT-116 | | | | | | | MDA-MB-231 | | | | | | |
| | 0.468 | 0.568 | 0.617 | 0.576 | 0.441 | 0.456 | 0.234 | 1.462 | 1.254 | 1.221 | 1.314 | 1.21 | 1.244 | 1.209 |
| | 0.419 | 0.502 | 0.666 | 0.355 | 0.449 | 0.316 | 0.306 | 1.407 | 1.068 | 1.017 | 1.116 | 1.062 | 1.293 | 1.283 |
| | 0.381 | 0.499 | 0.591 | 0.618 | 0.5 | 0.398 | 0.252 | 1.128 | 1.315 | 1.121 | 1.086 | 1.267 | 1.238 | 1.222 |
| | 0.387 | 0.507 | 0.6 | 0.433 | 0.319 | 0.362 | 0.363 | 1.389 | 1.12 | 1.222 | 1.163 | 1.204 | 1.246 | 1.218 |
| | 0.553 | 0.583 | 0.488 | 0.555 | 0.377 | 0.282 | 0.312 | 1.541 | 1.192 | 1.323 | 1.353 | 1.311 | 1.3 | 1.078 |
| | 0.692 | 0.545 | 0.386 | 0.474 | 0.446 | 0.486 | 0.302 | 1.589 | 1.651 | 1.487 | 1.265 | 1.317 | 1.102 | 1.249 |
| | 0.443 | 0.553 | 0.397 | 0.49 | 0.421 | 0.361 | 0.289 | 1.622 | 1.282 | 1.399 | 1.20 | 1.224 | 1.203 | 1.234 |
| | 0.597 | 0.539 | 0.509 | 0.513 | 0.446 | 0.359 | 0.275 | 1.887 | 1.126 | 1.103 | 1.17 | 1.12 | 1.14 | 1.19 |
| | 0.642 | 0.557 | 0.541 | 0.503 | 0.399 | 0.404 | 0.321 | 1.418 | 1.391 | 1.2 | 1.19 | 1.20 | 1.18 | 1.12 |
| **72 h** | HCT-116 | | | | | | | MDA-MB-231 | | | | | | |
| | 2.115 | 1.232 | 1.8 | 1.994 | 2.223 | 0.977 | 0.246 | 2.03 | 2.041 | 2.017 | 1.892 | 2.265 | 2.049 | 1.259 |
| | 2.028 | 1.596 | 1.771 | 1.64 | 1.292 | 0.784 | 0.246 | 1.944 | 1.952 | 1.962 | 1.789 | 1.795 | 1.916 | 1.661 |
| | 2.191 | 1.865 | 1.732 | 1.974 | 1.515 | 0.868 | 0.291 | 2.219 | 1.738 | 2.101 | 1.898 | 1.609 | 1.903 | 1.409 |
| | 2.201 | 1.967 | 1.945 | 1.787 | 1.341 | 0.822 | 0.276 | 1.669 | 2.057 | 2.251 | 1.977 | 1.957 | 2.01 | 1.557 |
| | 2.216 | 2.216 | 1.997 | 1.912 | 1.119 | 0.52 | 0.239 | 2.158 | 1.933 | 1.931 | 2.116 | 2.13 | 1.865 | 1.807 |
| | 1.608 | 2.028 | 1.802 | 1.599 | 1.618 | 0.777 | 0.227 | 1.93 | 1.909 | 2.106 | 2.157 | 2.029 | 1.907 | 1.603 |
| | 2.35 | 2.075 | 1.719 | 1.823 | 1.495 | 0.795 | 0.202 | 1.826 | 1.989 | 1.89 | 1.993 | 1.875 | 1.865 | 1.573 |
| | 1.526 | 1.914 | 1.813 | 1.787 | 1.52 | 0.815 | 0.292 | 2.027 | 2.281 | 1.921 | 1.921 | 1.89 | 1.922 | 1.506 |
| | 2.1 | 1.862 | 1.822 | 1.899 | 1.539 | 0.765 | 0.269 | 1.807 | 2.251 | 1.818 | 2.031 | 2.011 | 2.109 | 1.569 |

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**Supplementary Table 3:** The effect of HEAE on migratory potential of HCT-116 cells and MDA-MB-231 presented as measurements of wound space by ImageJ software (Raw data concerning **Figures 3** and **5**)

| Time of treatment | Concentration (µg/mL) | | | | | |
|---|---|---|---|---|---|---|
| | control *0* | *10* | *100* | control *0* | *10* | *100* |
| **0 h** | | HCT-116 | | | MDA-MB-231 | |
| | 1.237 | 1.363 | 1.285 | 1.28 | 1.155 | 1.356 |
| | 1.283 | 1.453 | 1.295 | 1.253 | 1.155 | 1.201 |
| | 1.064 | 1.245 | 1.295 | 1.335 | 1.146 | 1.301 |
| | 1.41 | 1.326 | 1.358 | 1.317 | 1.128 | 1.228 |
| | 1.283 | 1.099 | 1.213 | 1.371 | 1.137 | 1.365 |
| | 1.228 | 1.317 | 1.213 | 1.317 | 1.283 | 1.237 |
| **12 h** | | HCT-116 | | | MDA-MB-231 | |
| | 0.628 | 0.581 | 0.893 | 0.036 | 0.055 | 0.055 |
| | 0.582 | 0.6 | 1.003 | 0.055 | 0.055 | 0.064 |
| | 0.664 | 0.536 | 0.784 | 0.045 | 0.055 | 0.055 |
| | 0.619 | 0.636 | 0.866 | 0.054 | 0.073 | 0.045 |
| | 0.573 | 0.627 | 0.839 | 0.036 | 0.046 | 0.055 |
| | 0.682 | 0.636 | 0.811 | 0.036 | 0.046 | 0.038 |
| **24 h** | | HCT-116 | | | MDA-MB-231 | |
| | 0.246 | 0.409 | 0.647 | 0.029 | 0.036 | 0.036 |
| | 0.309 | 0.391 | 0.702 | 0.009 | 0.027 | 0.029 |
| | 0.309 | 0.409 | 0.693 | 0.029 | 0.055 | 0.027 |
| | 0.209 | 0.391 | 0.611 | 0.027 | 0.027 | 0.029 |
| | 0.246 | 0.3 | 0.638 | 0.018 | 0.027 | 0.036 |
| | 0.3 | 0.372 | 0.729 | 0.027 | 0.036 | 0.027 |

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**Supplementary Table 4:** Number of HCT-116 and MDA-MB-231 cells observed after HEAE exposure (in concentrations 100 and 250 µg/mL) (Raw data concerning **Figure 4**)

| | Viable cells | Early apoptotic cells | Late apoptotic cells | Necrotic cells | ∑ |
|---|---|---|---|---|---|
| | | | HCT-116 | | |
| | | | **24 h** | | |
| **0 µg/mL** | 445 | 5 | 0 | 0 | 450 |
| | 305 | 4 | 0 | 0 | 309 |
| **100 µg/mL** | 223 | 98 | 34 | 4 | 356 |
| | 193 | 82 | 29 | 3 | 307 |
| **250 µg/mL** | 261 | 160 | 116 | 12 | 549 |
| | 203 | 125 | 90 | 9 | 427 |
| | | | **72 h** | | |
| **0 µg/mL** | 534 | 22 | 0 | 0 | 556 |
| | 422 | 17 | 0 | 0 | 439 |
| **100 µg/mL** | 261 | 120 | 140 | 16 | 537 |
| | 243 | 112 | 132 | 15 | 502 |
| **250 µg/mL** | 97 | 111 | 92 | 98 | 398 |
| | 98 | 112 | 93 | 98 | 401 |
| | | | MDA-MB-231 | | |
| | | | **24 h** | | |
| **0 µg/mL** | 421 | 4 | 0 | 0 | 425 |
| | 318 | 3 | 0 | 0 | 321 |
| **100 µg/mL** | 190 | 129 | 35 | 1 | 355 |
| | 208 | 142 | 39 | 0 | 389 |
| **250 µg/mL** | 154 | 88 | 56 | 8 | 306 |
| | 159 | 91 | 58 | 8 | 317 |
| | | | **72 h** | | |
| **0 µg/mL** | 499 | 11 | 0 | 0 | 510 |
| | 314 | 7 | 0 | 0 | 321 |
| **100 µg/mL** | 325 | 69 | 16 | 0 | 410 |
| | 294 | 63 | 14 | 0 | 371 |
| **250 µg/mL** | 232 | 84 | 29 | 0 | 345 |
| | 282 | 102 | 35 | 0 | 419 |

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**Supplementary Table 5:** Observed absorbances of effects of HEAE in DPPH radical scavenging activity, superoxide anion scavenging activity, reducing power, and phenolics content (Raw data concerning **Table 2**)

**DPPH radical scavenging activity (Absorbance 517 nm)**

| | Control | *Hygrophorus eburneus* | | | | | | Ascorbic acid | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (µg/mL) | *0* | *1000* | *500* | *250* | *125* | *62.5* | *31.2* | *100* | *50* | *25* | *12.5* | *6.25* | *3.12* |
| n1 | 1.0270 | 0.0256 | 0.0307 | 0.3203 | 0.4590 | 0.5782 | 0.7415 | 0.0239 | 0.0454 | 0.1471 | 0.3904 | 0.6497 | 0.8164 |
| n2 | 1.0936 | 0.0297 | 0.0395 | 0.2996 | 0.4983 | 0.5995 | 0.8298 | 0.0254 | 0.0609 | 0.1572 | 0.4199 | 0.6989 | 0.8727 |
| n3 | 1.0553 | 0.0291 | 0.0361 | 0.3009 | 0.4889 | 0.5895 | 0.7695 | 0.0251 | 0.0845 | 0.1727 | 0.3946 | 0.6793 | 0.8184 |

**Superoxide anion scavenging activity (Absorbance 560 nm)**

| | Control | *Hygrophorus eburneus* | | | | | | Ascorbic acid | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Concentration (µg/mL) | *0* | *1000* | *500* | *250* | *125* | *62.5* | *31.2* | *1000* | *500* | *250* | *125* | *62.5* | *31.2* |
| n1 | 0.7553 | 0.0411 | 0.1232 | 0.1931 | 0.2991 | 0.4242 | 0.6095 | 0.0401 | 0.1224 | 0.1928 | 0.2992 | 0.4144 | 0.6019 |
| n2 | 0.7596 | 0.0391 | 0.1259 | 0.1983 | 0.3005 | 0.4251 | 0.6102 | 0.0388 | 0.1251 | 0.1981 | 0.2095 | 0.4219 | 0.6092 |
| n3 | 0.7591 | 0.0389 | 0.1257 | 0.1985 | 0.3011 | 0.4241 | 0.6117 | 0.0391 | 0.1260 | 0.1908 | 0.2995 | 0.4203 | 0.6120 |

**Reducing power (Absorbance 700 nm)**

| | *Hygrophorus eburneus* | | | | Ascorbic acid | | | |
|---|---|---|---|---|---|---|---|---|
| Concentration (µg/mL) | *1000* | *500* | *250* | *125* | *1000* | *500* | *250* | *125* |
| n1 | 0.0971 | 0.0699 | 0.0381 | 0.0299 | 2.1475 | 1.6509 | 0.9611 | 0.4174 |
| n2 | 0.1511 | 0.0401 | 0.0312 | 0.0206 | 2.1001 | 1.6719 | 0.9696 | 0.4199 |
| n3 | 0.1001 | 0.0404 | 0.0433 | 0.0201 | 2.0998 | 1.6301 | 0.9521 | 0.4091 |

**Phenolics content (Absorbance 760 nm)**

| | |
|---|---|
| n1 | 0.0361 |
| n2 | 0.0349 |
| n3 | 0.0381 |