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SHORT COMMUNICATION

Antioxidant and hyaluronidase inhibitory activities of diverse phenolics in *Phyllanthus emblica*

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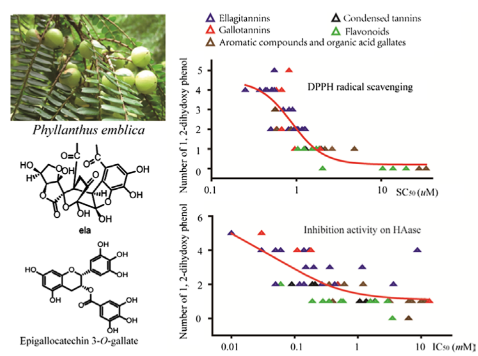
ABSTRACT
Fifty-eight phenolic compounds isolated from *Phyllanthus emblica* were screened and compared for their *in vitro* and *in vivo* antioxidant properties, as well as hyaluronidase (HAase) inhibitory activities. Among them, 20 compounds showed to be promising antioxidants due to the stronger scavenging activity in both DPPH radical and *Danio rerio* reactive oxygen species assays, while nine compounds were potential HAase inhibitors with 100-fold stronger activities than that of the positive control, DSCG. The structure activity relationship was discussed.



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1. Introduction

Phyllanthus emblica L. (Euphorbiaceae), a fruit-bearing tree widely growing in the southern China, India, and Southeast Asia, has been used in many traditional medicinal systems, and its fruits are consumed freshly or processed into food products in its growing areas

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(Calixto et al. 1998). Various chemical constituents have been reported from the titled plant; (Habib-ur-Rehman et al. 2007; El-Desouky et al. 2008; Lv et al. 2014, in supplementary material), and a wide range of bioactivities of *P. emblica* were associated with its phenolic constituents (Guo et al. 2013; Gaire & Subedi 2016). However, comprehensive and systemic evaluations, particularly the *in vivo* antioxidant activity on diverse phenolics from *P. emblica*, are unavailable. Herein, we report the inhibitory activity on hyaluronidase (HAase) and antioxidant activity in DPPH radical and *Danio rerio* reactive oxygen species (ROS)-scavenging assays of 58 phenolics from *P. emblica*. The structure activity relationship of the phenolics was discussed.

2. Results and discussion

2.1. Compounds from P. emblica

Fifty-eight phenolics isolated previously from *P. emblica* by our group comprised of five structural types, ellagitannins (**1**–**18**), gallotannins (**19**–**25**), simple phenolics (**25**–**35**), condensed tannins (**36**–**44**), and flavonoids (**45**–**58**) (Figures S1 and S2).

2.2. HAase inhibition of phenolics from P. emblica

Fifty compounds (**1**–**24**, **28**–**33**, **35**–**45**, **47**–**54**, **57)** were tested for the HAase inhibitory activity (Table S1 and Figure S3(a–e)). Eight hydrolysable tannins (**2**, **4**–**6**, **10**, **14**–**15**, **22**) and one dihydroflavone (**45**) with a galloyl group in molecules showed the strongest activity (IC_{50} < 0.09 mM), almost 100-fold stronger than that of the positive control (DSCG, IC_{50} = 6.99 ± 0.78 mM). The order of their inhibitory ability was **22** > **2** > **6** > **5** > **10** > **15** > **4** > **14** > **45**.

According to the structural types, the HAase inhibitory ability of phenolics in *P. emblica* was ellagitannins > condensed tannins > flavonoids > gallotannins > simple phenolics (Figure S3(f)). As the major phenolics in *P. emblica*, ellagitannins **1**–**18** showed mostly the strongest activity. All gallotannins (**19**–**25**) also showed stronger activities than the positive control, except **24** with the fewest free hydroxyl group in molecule. Among the simple phenolics **28**–**33** and **35**, only **28** displayed stronger activity (IC_{50} = 0.61 mM) than positive control. All the condensed tannins **36**–**44** and flavonoids **45**, **47**–**54**, and **57** showed stronger activities than positive control. Among which, **45** showed the strongest activity (IC_{50} = 0.09 mM). The aforementioned results suggested that the HAase inhibition assay showed a clear correlative relationships (Figure S3(g–h)) between the phenol, especially *ortho*-dihydroxyphenol numbers. For example, the activity order of **6** > **3** > **9** > **13** was accordant with the phenol and *ortho*-dihydroxyphenol numbers. The one with more *ortho*-dihyroxyphenol units exhibit stronger activity. On the other hand, more free carboxyls in molecules decreased the inhibition effects on HAase, e.g. compounds **1** and **25**–**35**.

2.3. DPPH radical-scavenging activity of phenolics from P. emblica

The DPPH radical-scavenging activities of **1**–**58** were shown in Table S1 and Figure S4(a–e). The ellagitannins exhibited the strongest activity. For example, **1**–**4** showed roughly six-fold stronger activities than positive control. Gallotannins (**19**–**25**) and condensed tannins (**36**–**44**) also displayed stronger activities than positive control. Of them, the gallotannins (**19**–**21**) with more free hydroxyl groups showed the strongest activities. All simple phenolics **25**–**35** showed the lowest activities, due to less or no *ortho*-dihydroxyphenol unit in molecules. Flavonoids (**45**–**58**) displayed the same radical-scavenging ability with positive control. Among them, **45**–**47** with galloyl group showed the strongest activities. The above results indicated a clear correlative relationship in DPPH radical-scavenging assay (Figure S4(g–h)). For example, the less phenol and *ortho*-dihydroxyphenol numbers (**8** < **7** < **1** < **6**), the weaker DPPH radical-scavenging ability it have. This was consistent with that reported in the literatures (Wang et al. 2007), indicating that the antioxidant activity of phenolics decreased when less numbers of *ortho*-dihydroxyphenol units existed in molecules.

2.4. *Danio rerio ROS-scavenging activity of phenolics from* P. emblica

All of the isolates **1**–**58** were tested for their individual antioxidant activities in zebrafish model (Table S1 and Figure S5(a–e)). Most tested phenolics displayed potential activity in ROS-scavenging assay. At a concentration of 30 μM, 15 compounds with an activity order of **46** > **8** > **41** > **56** > **44** > **42** > **51** > **53** > **2** > **49** > **55** > **58** > **45** > **57** > **35** showed more than 100% ROS clearance rates. Ten of them (**44**–**46**, **49**, **51**, **53**, **55**–**58**) were flavonoids, which showed weaker activities than ellagitannins in DPPH radical assay. Most ellagitannins and gallotannins with promising DPPH radical-scavenging activities showed no activity in the ROS-scavenging assay, except ellagitannins **2** and **8**. The order of the ROS clearant ability of phenolics in *P. emblica* was flavonoids > simple phenolics > condensed tannins > gallotannins > ellagitannins (Figure S5(f)). The aforementioned observation showed that, unlike DPPH radical-scavenging activity, the *in vivo* antioxidant activity of phenolic compounds has no direct relationship with their free phenol or *ortho*-dihydroxyphenol units. However, ellagitannins with less free carboxyl groups showed stronger ROS-scavenging abilities. This is consisted with DPPH radical-scavenging activity. Moreover, ellagitannins **2** and **8** with an elaeocarpusinosyl (Ela) ester group, showed the strongest ROS-scavenging activity, indicating that the Ela fragment should be a good scaffold for the *in vivo* ROS-scavenging activity. The condensed tannins with *ortho*-trihydroxyphenol exhibited stronger ROS-scavenging activities than the ones with *ortho*-dihydroxyphenol, and the dimers **37**–**38** and **40** exhibited weaker activity than the monomers (**36**, **42**).

3. Conclusion

Most of the phenolics in *P. emblica* showed potential antioxidative and HAase inhibitory activities. Six ellagitannins (**2**–**3**, **7**–**8**, **13**, **17**), one gallotannin (**19**), two simple phenolics (**31**, **33**), four condensed tannins (**36**, **41**–**42**, **44**), and six flavonoids (**45**–**46**, **49**, **51**, **53**, **55**, **58**) are promising antioxidants, due to their stronger activities in both DPPH radical and *in vivo* ROS-scavenging assays. Seven ellagitannins (**2**, **4**–**6**, **10**, **14**–**15**), one gallotannin (**22**) and one flavonoid (**45**) were potential HAase inhibitors, due to their 100-fold stronger activities than positive control. The numbers of free phenols and *ortho*-dihydroxyphenols in molecules increased the activity of phenolics in both HAase and DPPH assays. It is also noted that the existence of more free carboxyl groups in phenolics would impair their antioxidant activities *in vivo* and *in vitro*. With the existence of Ela ester group, the phenolics displayed stronger antioxidant potential *in vivo* ROS-scavenging assay, suggesting Ela unit should be a good scaffold for antioxidative properties. The present study supported that *P. emblica* represents a valuable natural source of antioxidants and HAase inhibitors with useful potential for food, cosmetic and pharmaceutical industries.

Supplementary material

Supplementary material relating to this article is available online: experimental part, structures of compounds **1**–**58** (Figures S1–S2), HAase inhibitory, DPPH radical and *Danio rerio* ROS-scavenging activities of **1**–**58** (Figures S3–S5 and Table S1).

Disclosure statement

No potential conflict of interest was reported by the authors.

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