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Progress in studying the bioactive functions of Phyllanthus emblica L.

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Abstract:

Phyllanthus emblica L., belonging to the Phyllanthaceae family, is extensively distributed across tropical and subtropical regions. PE exhibits a range of therapeutic properties, including anti-inflammatory, antioxidant, and neuroprotective effects. It is laden with a high concentration of various bioactive compounds such as phenolic acids, flavonoids, and terpenoids. Notably, flavonols are among the most copious flavonoids present in PE fruit. Its potent antioxidant activity is primarily attributed to its high content of polyphenols, which comprise 45% of the dry fruit's mass.Polyphenols in PE possess the capability to neutralize free radicals or chelate iron ions and are considered as good antioxidants. The phytochemicals such as tannins and terpenoids in PE are antibacterial and can act as natural antimicrobial agents to inhibit bacterial growth by destroying the bacterial cell wall or preventing the synthesis of key enzymes. Furthermore, PE possesses notable anti-inflammatory attributes, mitigating inflammation through the suppression of critical enzymes integral to the inflammatory cascade, including COX-1, COX-2, and 5-LOX. The antioxidant, antimicrobial, and anti-inflammatory characteristics of PE render it a promising candidate for a variety of applications within the realms of food and medicine.

Keywords: *Phyllanthus emblica L.*, antioxidation, bacteriostasis, anti-inflammatory

1 Introduction

Phyllanthus emblica L. (PE), a species belonging to the Phyllanthus genus within the Euphorbiaceae family as classified by Jussieu, is variously referred to as balakka, kimalaka, kemlaka, kemloko, or malaka in Indonesia[1]. There are about 600 species of PE in the world,and most of them can be found in tropical and subtropical regions. Moreover, approximately thirty species are predominantly found in regions south of the *Yangtze River* in China. Historically, PE was initially utilized within the domain of conventional Tibetan medicine in China, and it was among the pioneering substances to be integrated into the Pharmacopoeia of the People's Republic of China, recognized for its dual utility in both medicinal and culinary contexts. Comprehensive documentation has attested to the therapeutic attributes of PE, which encompass anti-inflammatory, antioxidant, and neuroprotective effects[2].

Natural antioxidants refer to active substances extracted from natural products with antioxidant capacity, which have the biological property of scavenging or inhibiting oxygen free radicals[3]. Phenolic acids was key bioactive components with pharmacological activity and strong antioxidant activity. In addition to phenolic acids, PE is also composed of flavonoids, terpenes, and other bioactive constituents. Notably, flavonols represent one of the most prevalent subclasses of flavonoids within the fruit of PE, with a majority being present in the form of glycosides[4]. These active components in PE had good DPPH scavenging capacity and the ability to scavenge superoxide anions, hydroxyl radicals and chelated iron, thereby achieving their antioxidant functions[5].

However, due to its sour and astringent entrance, in the food field, except for the production of a small number of beverages, the rest of the value had yet to be explored. Studies had shown that the removal of bitter substances from *Phyllanthus emblica* did not affect its antioxidant activity[6].

2 phytochemicals

2.1 Polyphenols

Phenolics constitute a class of secondary metabolites that are endogenously produced within plants under standard growth conditions and during developmental processes, as well as in response to a multitude of biotic and abiotic stressors. This class comprises a wide spectrum of phytochemical entities, which vary from compounds with rudimentary molecular structures to those characterized by intricate and sophisticated configurations[7].Based on their distinct structural characteristics, polyphenols can be categorized into three primary groups: flavonoids, phenolic acids, and tannins.

Phenolic acids account for about 1/3 of the total phenols in plant-derived foods, and most of them are benzoic acid, cinnamic acid and their hydroxylated derivatives[8]. Investigations have demonstrated that phenolic acids constitute the predominant class of phenolic compounds within the fruit of PE, encompassing 10 hydroxycinnamic acids and 15 hydroxybenzoic acids[9]. It has been documented that compounds such as caffeic and protocatechuic acids, characterized by straightforward dihydroxylation structures, demonstrate superior antioxidant properties. Conversely, 4-hydroxybenzoin and 4-Hydroxycinnamic acid, which possess molecules with mono-hydroxylation structures, exhibit the least potent antioxidant effects across numerous assays. The enhanced antioxidant efficacy of 3,4-Dihydroxycinnamic acid over 3,4-dihydroxybenzoic acid, and 4-Hydroxy-3-methoxycinnamic acid over 3-Hydroxy-4-methoxycinnamic acid, can likely be ascribed to the inherent properties and the strategic positioning of substituents on their respective benzene rings. The antioxidant activities of caftaric acid, dicaffeoyltartaric acid, and 3-O-Caffeoylquinic acid, along with their derivatives featuring complex dihydroxylation structures, were comparatively high within the realm of phenolic acids[10]. As report, phenolic substances had been extracted from PE. Such as Caffeine Acid, 2-O-Caffeoyl Hydroxycitric Acid, and Caffeic 3-O-Glucuronic Acid, 1-ethylacid-3 oxycarbamate, 6-methylester-3-oxycarbamate, and 6-ethylester-2-oxoformate[9]. The content of phenolic acids in PE could reach (0.2427±0.0080) mg/GAE·mg^{-1}, Most of them were distributed in the fruits, included 15 kinds of 4-Hydroxybenzoic acid and 10 species of p-Hydroxy-cinnamic acid[2].

Flavonoids are polyphenolic compounds with C6-C3-C6 basic framework, and their basic structure includes two aromatic rings and one pyran ring, which could be divided into flavonoids, flavonols, flavanols, dihydroflavonols, anthocyanins and isoflavones according to their structure. Flavonols represent one of the most copious subclasses of flavonoids present in the fruit of PE, with a majority being identified in the glycoside form, a configuration that facilitates their binding to the cell wall[9]. The presence of various flavonoids has been observed, including notable compounds such as kaempferol-3-O-α-L-(6'-ethyl)-rhamnopyranoside, quercetin, acylated apigenin glucoside, and kaempferol-3-O-α-L-(6'-methyl)-rhamnopyranoside, among others[2].

Tannins, also known as tannic acid, are a class of complex polyphenolic compounds. In light of their structural attributes, tannins can be categorized into two distinct types: hydrolyzed tannin and condensed tannins. Hydrolyzed tannins, also known as gallic acid, includ esters composed of phenolic acid and polyol derived from gallate. The intramolecular ester bonds are susceptible to hydrolysis in the presence of acid, enzyme, or alkali, yielding polyols and phenolic acids; condensed tannanins were flavanol derivatives, and flavanol in the molecule was bound to catechols or phenol through the second C=C bond[11]. Hydrolyzed were found in *P. emblica* including punigluconin, Emblicanin, pedunculagin, isochorylagin, geranin, chebulanic acid, corylagin, chebulagate acid, gallic acid, isostrictinin, digalloylglucose, methyl gallate, among others[2].

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2.2 Terpenoids

Terpenoids represent one of the most extensive and structurally varied categories of naturally occurring compounds. They are a class of natural products arise from the biosynthetic pathway of mevalonic acid (MVA), each molecule being assembled from multiple isoprene (C5) structural units. PE harbors chemopreventive compounds such as lupol acetate and glochidone. Furthermore, the substance possesses notable antioxidant attributes, which are attributed to compounds such as isostrictinin, mallonin, and mallotusinin, in conjunction with cinnamic acid, chebulagic acid, and phyllembilin. Studies show that the terpenoids are mostly concentrated in the root of PE[12].

3 Active function

3.1 Antioxidation

The relationship between oxidative stress and the balance of free radicals and antioxidants is well-documented. Oxidative stress occurs when there is an imbalance between the production of free radicals and the availability of antioxidants, often leading to an augmented generation of free radicals or a diminished level of antioxidants. The initiation of oxidative stress happens when the equilibrium between pro-oxidant and antioxidant entities is disrupted. Both pro-oxidants and free radicals are characterized by the presence of multiple unpaired electrons, a feature that makes them inherently unstable and excessively reactive with other substances[2]. The antioxidant function within the PE stems from its abundance of polyphenols. The polyphenols in PE represent about 45% of their dried fruit mass[13]. One study showed that the contents of gallate, chlorogenate, lagistase and quercetin extracted coming from PE juice were 37.95, 17.43, 71.20 and 2.01 mg / 100 ml, respectively. beta-Glucogallin; 1,6-O-Digalloyl-β-D-glucopyranose; 3,6-di-*O*-galloyl-d-glucose; gallate; chebulinic acid; quercitroside; chebulagic acid; corillagin; 3-ethylgallic acid(3-ethoxy-4,5-dihydroxy-benzoic acid); isostrictiniin was found in juice of PE as well[14]. In addition, many new polyphenols have been detected in recent years. In 2023, Sun et al. isolated three new phenolic substances in PE: 1-ethyl-3-*O*-formate, 6-methyl-3-*O*-formate and 6-ethyl-2-oxyformate. 3,3'-Dimethoxy ellagic acid-4'-O-β-D-glucoside and (E)-1-O-β-D-glucoside were initially isolated from PE[15].

Most of them could inhibit oxidative stress by scavenging free radicals or chelating iron ions, so they are regarded as good antioxidants. The distribution of different antioxidants in PE varies, which is shown by the different antioxidant capacity of parts of PE. Gallic acid is the exclusive hydroxybenzoic acid that has been documented within the leaves and branches. The occurrence of hydroxycinnamic acids, specifically caffeic acid and chlorogenic acid, has been observed exclusively in amla fruits[16]. Analytical data reveal that the phenolic concentration is higher in the pulp, whereas tannins are the principal constituents within the seeds of PE. Compounds such as coumaric acid, myricetin, caffeic acid, and synergic acid have been detected in both the pulp and the seeds. In contrast, gallic acid and quercetin are found exclusively in the pulp and are absent in the seeds of PE[14]. One investigation demonstrated that the cumulative phenolic concentration within the fruits and foliage of Pyrus etrusca was markedly superior to that observed in its stems. The fruits had the highest content of flavonoids, followed by leaves and the least stem branches. The tannic acid content was highest in shoot branches, followed by leaves and fruits[15]. In addition, another study showed that the total phenol, tannin and flavonoids in the seeds were 4.53 ± 0.18mg GAE/g, 126.71 ± 0.92mg TAE/g, 1016.25 ± 16.50mg QE/g, while the three fruits were 6.00 ± 0.18mg GAE/g, 52.96 ± 1.04mg TAE/g and 865 ± 34.28mg QE/g. Tannins and flavonoids were significantly higher in seeds than in fruits[17]. Notwithstanding the elevated cumulative phenolic content within the fruit as opposed to the seeds, the latter demonstrated a superior capability in scavenging free radicals. This enhanced efficacy is presumably attributable to the predominant composition of free polyphenols, predominantly represented by flavonoids, which play a pivotal role in their antioxidative properties.

Polyphenols could also avoid the oxidative degradation of lipids by reducing iron ions and inhibiting the $FeCl_3$-ascorbate-mediated lipid peroxidation[18]. By the comparison of antioxidant activity of different PE extracts, Li et al found that the highest antioxidant activity and the best iron reduction ability of PE95% ethanol extract[18].

3.2 Bacteriostasis

Phytochemicals like Tannins, terpenoids in PE have good antibacterial effects and could be used as natural antibacterial agents. Natural antibacterial agents pertain to a class of substances, characterized by intricate molecular structures, which are derived from the bounties of nature via extraction from animals and plants, or else synthesized through microbial metabolic processes. The antibacterial substances in plants are mainly some bioactive ingredients in essential oil, which are characterized by non-toxic, high efficiency and wide antibacterial spectrum[19]. The inhibition of phytochemicals in PE is mainly achieved by destroying the bacterial cell wall or preventing the synthesis of key enzymes. It is reported that the realization of the antibacterial function of plant polyphenols is accomplished by blocking the information expression and communication of bacteria. By blocking bacterial communication, it could inhibit colony sporulation and virulence factors. Such a manner does not kill the strain, so a particular advantage of using polyphenol inhibition is that no resistance will develop[20]. The polyphenolic compounds referred to as catechins have been noted to increase the permeability of the cell membrane in Escherichia coli by disrupting the synthesis of RpoS and OmpC proteins. These proteins are essential for the preservation of the structural integrity and functional efficacy of the bacterial envelope[21]. Certain anthocyanins have the capability to suppress the expression of the vtx 1 and vtx 2 genes in Escherichia coli. This disruption compromises the cell membrane in E. coli O157:H7, leading to cytoplasmic leakage. This leakage ultimately manifests as a bacteriostatic effect, inhibiting the growth of the bacteria[22]. Tea polyphenols possess the ability to impede the motility of Klebsiella pneumoniae as well as to suppress its biofilm formation. Additionally, they can decrement the production of extracellular polymeric substances and proteases[21].

The inhibitory principle of terpenoids in PE is the inhibition of cell membrane or cell wall synthesis[23]. Banerjee and colleagues discovered that andrographolide demonstrated notable antibacterial efficacy against the majority of the evaluated Gram-positive bacteria. Notably, it exhibited the highest sensitivity towards Staphylococcus species and was observed to effectively inhibit the biofilm formation of Staphylococcus aureus[23]. PE exhibits a notably higher antibacterial efficacy toward Gram-positive bacteria, in contrast to its comparative ineffectiveness against Gram-negative strains. It may be because the seeds contains more terpenoids.

In recent years, some scientists have made a new attempt in the field of PE bacteriostasis research, combining it with biological metals to form nanoparticles and study their antibacterial effect. Nanocomplex Se-NPs synthesized by PE and selenium were reported to effectively reduce the survival rate of Staphylococcus aureus in meat products, and the main reason of their antibacterial effect is NPs have the potential to compromise the integrity of bacterial cell membranes, capable of penetrating the cell wall/membrane barrier. They can interact with and disrupt the DNA structure. Furthermore, it serves to catalyze an escalation in the generation of reactive oxygen species within the bacterial cellular environment. These combined effects may lead to microbial cell lysis and death[24].

3.3 Antiinflammatory

Inflammation plays a pivotal role in the organism's innate immune response, acting as a crucial defense mechanism against exogenous irritants. The initiation of inflammation is precipitated by the secretion of proinflammatory cytokines, such as interleukin 6, interleukin 8, and tumor necrosis factor alpha, in conjunction with the production of reactive oxygen species[25]. Inflammatory responses are considered to be advantageous pathological phenomena, owing to their pivotal function in orchestrating recuperative, healing, and invasive processes, especially in relation to the resistance against stressors elicited by pathogens and deleterious conditions. Inflammatory responses are distinctly classified into acute and chronic inflammation. Acute inflammation functions as a safeguard for the organism, whereas chronic inflammation directed at vital cells, molecules, and organs contributes to the initiation and exacerbation of diverse chronic diseases.

The anti-inflammatory effects of PE are exerted through mechanisms that include the inhibition of pivotal enzymes integral to inflammatory processes, specifically COX-1, COX-2, and 5-LOX. These enzymes play a crucial role in the synthesis of proinflammatory mediators, and their inhibition is essential for alleviating inflammation[2]. Enzymatic activities, such as those of COX-2, result in the production of nitric oxide (NO). Nitric oxide serves as a critical molecule within the immune-signaling pathway. Excessive production of NO can precipitate inflammatory responses. Consequently, PE precludes the synthesis of NO by COX-2 enzyme through the suppression of its enzymatic activity[26].

4 Conclusion

Phyllanthus emblica (PE), belonging to the *Euphorbiaceae* family, is primarily distributed in tropical and subtropical climates, featuring an especially abundant presence in the southern reaches of the *Yangtze River Valley* in China. This plant exhibits a diverse array of biological activities, encompassing anti-inflammatory, antioxidant, and bacteriostatic properties. The fruits of PE are replete with an array of natural antioxidants, including phenolic acids, flavonoids, and terpenoids, with flavonols being among the most prevalent flavonoids present in these fruits. The antioxidative characteristics of PE are predominantly ascribed to its substantial concentration of polyphenolic substances, composing 45% of the dried fruit's mass. These compounds possess the efficacy to neutralize free radicals and sequester iron ions, thereby manifesting their antioxidative influences. Moreover, phytochemicals such as tannins and terpenoids in PE have antimicrobial effects, and they

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can inhibit bacterial growth by disrupting the bacterial cell wall or inhibiting the synthesis of key enzymes. PE also exhibits anti-inflammatory properties by inhibiting the activity of pivotal enzymes, such as COX-1, COX-2, and 5-LOX, which play significant roles in the inflammatory cascade. Owing to its antioxidant, antimicrobial, and anti-inflammatory attributes, PE holds considerable promise for applications in the food and pharmaceutical industries.

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