Isolation Of *P*-Hydroxy Benzoic Acid from *Holarrhena Antidysenterica* (L.) And Its Conversion to Value-Added Derivatives

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***Abstract*—*p*-Hydroxybenzoic acid is reported to possess diverse biological properties, including antimicrobial, antioxidant, anti-inflammatory, antithrombogenic and anticancer activities. In addition, it has been associated with lowering liver and serum cholesterol levels, enhancing sperm viability, and offering benefits relevant to coronary heart disease. Beyond its therapeutic potential, this compound holds significant commercial value in the food, healthcare, and cosmetic industries. Since p-Hydroxybenzoic acid can be absorbed and metabolized within the human body, it provides further advantages. Considering these attributes, we designed a strategy to extract and isolate p-Hydroxybenzoic acid from *Holarrhena antidysenterica* (L.). Furthermore, based on its properties, we synthesized ester and amide analogues, which were subsequently characterized using ^{1}H NMR and GC-MS techniques.**

I. INTRODUCTION

Holarrhena antidysenterica (L.) Wall. ex DC. (Syn. *Echites antidysenterica* L.) is widely distributed across tropical and subtropical regions of Asia and Africa. This deciduous tree, ranging from small to medium size, is recognized for its characteristic bark, profuse flowering, and medicinal importance. The plant contains steroidal alkaloids such as conessine and has long been utilized in Ayurvedic and other traditional medical systems [1].

II. ROOTS

Holarrhena antidysenterica (L.) develops a deep, branched taproot system that enables access to water from lower soil layers. Young roots are light brown to yellowish-brown, gradually darkening with age due to secondary growth. The taproot, along with lateral branches extending horizontally and vertically, provides structural support and facilitates nutrient absorption. The roots are cylindrical, rough in texture, and marked with lenticels [2,3].

III. STEM

The main stem of *Holarrhena antidysenterica* (L.) is upright, woody, and cylindrical, branching frequently to form a rounded crown. The trunk diameter ranges between 20–30 cm, with a height of 3–12 meters. Young bark is thick, rough, and greyish to light brown, dotted with lenticels, while older bark becomes corky, cracked, and exfoliating. Initially greenish, the stem turns greyish-brown with maturity. The branching pattern, either dichotomous or sympodial, contributes to the tree's open canopy [4,5].

IV. LEAVES

Leaves are simple, opposite, and decussate, arranged in pairs at right angles. They are elliptic to ovate with a rounded base and tapering apex, measuring 5–10 cm in width and 10–20 cm in length. The upper surface is dark green and smooth, while the lower surface is lighter, sometimes bearing fine hairs along the midrib and veins in young leaves. Pinnate venation with prominent secondary veins is visible, and margins are entire. Petioles are short, typically 0.5–1 cm long [6].

V. FLOWERS

Flowers occur in axillary or terminal corymbose cymes, forming flat-topped inflorescences. They are small, radially symmetrical, and pentamerous, usually

white with occasional yellow tinges at the center. Each flower measures 2–3 cm in diameter. The calyx consists of five lanceolate sepals (2–4 mm), while the corolla is tubular with five spreading lobes, 1–1.5 cm long. Stamens are attached to the corolla tube, and the pistil comprises two carpels fused at the base, forming a superior ovary with a two-lobed stigma. Blooming generally occurs between April and July [7].

VI. FRUITS

The fruits are dry, dehiscent paired follicles that split along one side. Narrow and cylindrical, they taper at both ends, measuring 0.8–1.5 cm in width and 20–40 cm in length. Initially green, they turn brown upon maturity. Follicles occur in divergent pairs and open along a single suture to release seeds [8].

VII. SEEDS

Seeds are numerous, oblong, and winged. Each seed body is rectangular, measuring 0.3–0.5 cm in width and 1–2 cm in length, including the papery wing. The wing is whitish, while the seed body is light to dark brown. Seeds are distributed along the inner follicle surface and dispersed by wind after fruit dehiscence.

VIII. 4-HYDROXYBENZOIC ACID

4-Hydroxybenzoic acid (pHBA), a phenolic derivative of benzoic acid [9], appears as a white crystalline solid. It dissolves more readily in polar organic solvents such as acetone and alcohols compared to water or chloroform [10]. Its primary application is in the synthesis of esters (parabens), widely used as preservatives in ophthalmic solutions and cosmetics [11]. Structurally, it is isomeric with 3-hydroxybenzoic acid and 2-hydroxybenzoic acid (salicylic acid), the latter being a precursor of aspirin [12].

Natural sources of hydroxybenzoic acid include coconuts, green tea catechin metabolites, *Phyllanthus acidus* (Otaheite gooseberry), wine, vanilla, *Macrotyloma uniflorum* (horse gram), and carob [13]. High concentrations are found in açaí oil from *Euterpe oleracea* fruit (892±52 mg/kg), murky olive oil, and edible mushrooms such as *Russula virescens* [14]. It also occurs in *Vitex* species (*V. agnus-castus*, *V. negundo*), *Hypericum perforatum* (St. John's wort), freshwater algae (*Spongiochloris spongiosa*), and medicinal mushrooms like *Ganoderma lucidum*. Certain bacteria, such as *Cryptanaerobacter*, convert phenol to benzoate via 4-hydroxybenzoate [15]. Microalgae extracts (e.g., *Saccharina japonica*, *Odontella sinensis*, *Isochyrysis galbana*, *Phaeodactylum tricornutum*, *Chaetoceros calcitrans*, *Skeletonema costatum*) contain various phenolic acids, with *O. sinensis* showing the highest levels [16,17]. Hydroxybenzoic acids are also present in grasses and mushrooms, alongside hydroxycinnamic acids and other phenolic derivatives [18].

Plant cell walls predominantly contain hydroxycinnamates (C6–C3) and hydroxybenzoates (C6–C1), derived via the phenylpropanoid pathway [19]. Among these, p-hydroxybenzoic acid is particularly significant due to its industrial applications in food, cosmetics, and polymers [20]. It also serves as a precursor in the biosynthesis of pigments such as shikonin and quinones [21]. Although widespread in plants, the biosynthetic pathway leading to benzoates and hydroxybenzoates remains unclear [22].

Recent studies highlight the accumulation of p-hydroxybenzoic acid in *Daucus carota* hairy root cultures induced by *Agrobacterium rhizogenes*, with correlations observed between soluble and wall-bound phenolic acids [23]. Its incorporation into cell walls in an alkaline-labile form has also been reported [24]. Notably, 4-hydroxybenzoic acid exhibits estrogenic activity and promotes growth in human breast cancer cell lines both in vitro and in vivo. More recently, it has emerged as a valuable intermediate for bioproducts in food, cosmetics, pharmaceuticals, and fungicides. Advances in synthetic biology and metabolic engineering have enabled biosynthetic production of 4-HBA and its derivatives [25] In light of these properties, p-hydroxybenzoic acid was isolated from *Holarrhena antidysenterica* (L.).

IX. EXTRACTION PROCEDURE

Leaf material was finely powdered (2 kg) and subjected to Soxhlet extraction for 20 hours using 1000 ml of methanol. The resulting extract was concentrated under reduced pressure with a Rotavapour, yielding a viscous residue (25 g). This mass was suspended in 500 ml of distilled water and partitioned sequentially with 250 ml of hexane and 250

ml of ethyl acetate in a separatory funnel. After solvent removal under low pressure, 15 ml of hexane and 15 ml of ethyl acetate fractions were obtained.

The ethyl acetate fraction (15 ml) was further chromatographed on a Sephadex LH-20 column using methanol as the eluent. Thin-layer chromatography (TLC) monitoring revealed five major fractions, with p-hydroxybenzoic acid detected at Rf 0.41 in the solvent system toluene-ethyl acetate-formic acid (5:5:1). Fraction F-4 (200 mg), containing p-hydroxybenzoic acid, was purified by preparative TLC in the solvent system toluene–ethyl acetate–formic acid (10:10:1). Approximately 25 mg of F-4 was applied to a homemade preparative TLC plate (Silica gel GF254, 20 × 20 cm, 1 mm thickness). The band corresponding to p-hydroxybenzoic acid was scraped, and silica gel was washed with methanol. Evaporation of the solvent yielded white crystalline p-hydroxybenzoic acid (40 mg), which was finally purified and washed with hot water.

X. SYNTHESIS OF ANALOGUES

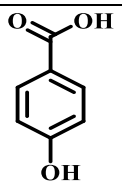
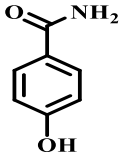
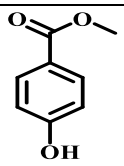
Amide Analogue Preparation

p-Hydroxybenzoic acid was reacted with $PC1_5$ (1:5) in a dry porcelain dish. The mixture was ground in a fume hood using a Morton pestle until liquefaction occurred, producing crude acid chloride. This intermediate was treated with concentrated NH_4OH, resulting in a vigorous reaction. After cooling, the product was filtered, washed with water, and recrystallized from aqueous methanol. The final compound obtained was 4-hydroxybenzamide, with a melting point of 226 °C.

Ester Analogue Preparation

p-Hydroxybenzoic acid was refluxed with methanol (1:1) in the presence of H_2SO_4 for 6–8 hours. The reaction mixture was worked up by washing with water, followed by distillation. The product obtained was methyl methyl 4-hydroxybenzoate, with a boiling point of 308–309 °C and a yield of 79%.

Table No. 1

Sr. No	Structure of Compound	Confirmational analysis by ^{1}HNMR and GCMS
1		^{1}HNMR (δ ppm): carboxylic -OH proton at 12.70 ppm, 9.65 at phenolic -OH, Ar-H protons at 6.90-7.91 ppm GCMS (m/z): 138.12 cal., Found 138.10
2		^{1}HNMR (δ ppm): phenolic -OH proton at 9.70 ppm, 8.40 ppm at amino -NH_2 and Ar-H protons at 6.88-7.70 ppm GCMS (m/z): 137.14 cal., Found 137.10
3		^{1}HNMR (δ ppm): phenolic -OH proton at 9.66 ppm, Ar-H protons at 6.81-7.75 ppm and CH_3 protons at 3.86 ppm GCMS (m/z): 152.15 cal., Found 152.12

XI. CONCLUSION

p-Hydroxybenzoic acid was successfully isolated and extracted from *Holarrhena antidysenterica* (L.), followed by the synthesis of ester and amide analogues that demonstrate wide-ranging potential across multiple fields. However, certain challenges remain, particularly in achieving higher efficiency and improved yields during analogue synthesis. This highlights the importance of developing medicinally active compounds, exploring diverse properties, and identifying novel applications of these analogues. Expanding research in this direction could open vast opportunities, especially within synthetic and applied sciences.

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Conflict of Interest
None

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