Review Article

# A Comprehensive Review on Biological Activities of P-Hydroxy Benzoic Acid and Its Derivatives

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**ABSTRACT**

p-hydroxy benzoic acid (PHBA) is an organic chemical which can be obtained naturally as well as synthetically. The literature survey reveals its various biological properties viz. antimicrobial, antialgal, antimutagenic, antiestrogenic, hypoglycemic, anti-inflammatory, anti-platelet aggregating, nematicidal, antiviral, antioxidant etc. It is also reported to be used as preservative in many drugs, cosmetic products, pharmaceuticals, food and beverages. Some derivatives of 4-hydroxybenzoicacid are found to possess direct action on Hbs molecules, inhibit acetic acid induced oedema and used in management of sickle cell disease. The present study will give comprehensive information of the biological activities of this p-hydroxy benzoic acid and its derivatives.

**Keywords:** PHBA, biological activities, antimicrobial.

## INTRODUCTION

4-hydroxy benzoic acid (4-hydroxy benzene carboxylic acid) having empirical formula $C_7H_6O_3$, molecular weight 138.13, melting point 216.2ºC is a biodegradable, less bioaccumulative organic solid chemical which can be isolated naturally from carrots (*Daucus carota*), oil palm (*Elaeis guineensis*), grapes (*Vitis vinifera*), east african satinwood (*Fagara macrophylla*), yellow leaf tree (*Xanthophyllum rubescens*), peroba (*Paratecoma peroba*), taheebo (*Tabebuia impetiginosa*), red sandalwood (*Pterocarpus santalinus*), southern catalpa (*Catalpa bognoniooides*), chinese chest tree (*Vitex negundo*), betel palm (*Areca catechu*), cuban royal palm (*Roystonea regia*) and medlar (*Mespilus germanica*) or can be synthesized chemically.[1] It is also detected in cell wall extracts of *Arabidopsis thaliana* roots and its concentration increased upon infection with *Pythium sylvaticum.* It is also synthesized de novo in stems and petioles in response to a mobile signal by *Pseudomonas syringae* pv. *Syringae.*[2]

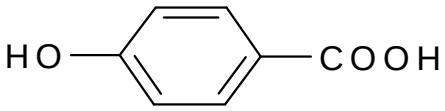


**Figure 1:** p-hydroxy benzoic acid

4-Hydroxy benzoic acid is reported to have antibacterial (against Gram +ve and Gram –ve bacteria), antifungal, antialgal, antimutagenic, antisickling and estrogenic activity. It is also used as trapping agent to study hydroxyl radical generation during cerebral ischemia and reperfusion and is also widely used as preservative in drugs, cosmetics, pharmaceuticals, in food and beverages.[3] It showed inhibitory effects on hepatic enzyme pyrophosphate decarboxylase and mevalonate phosphate kinase while competing with substrate mevalonate 5-pyrophosphate and also induces oxidative stress in the skin after conversion to glutathione conjugates of hydroquinone by reacting with singlet oxygen and glutathione.[4] Induction and control of p-hydroxybenzoic acid under stress conditions are important for the antioxidative system because biosynthesis of salicylic acid is catalyzed by benzoic acid 2-hydroxylase and connected with p-hydroxybenzoic acid.[5]

Horvath *et al*., 2007 reported that p-hydroxy benzoic acid increases abiotic stress tolerance of winter wheat (*Triticum aestivum* L.) and also increases the impermeability of the cell wall, leading to increased resistance against pathogen infection.[6] It has a growth stimulation effect on the freshwater green alga *Pseudokirchneriella subcapitata.*[7]

### Biological activities of p-hydroxy benzoic acid

### As Antimicrobial Agent:

The drastic increase of multidrug resistant microbial infections has become a serious health hazard. Medicinal chemists are always in a great research for new antimicrobial agents. 4-Hydroxy benzoic acid is an organic chemical that is reported to exhibit antimicrobial activity against a number of micro-organisms viz Gram positive as well as Gram negative bacteria like *Escherichia coli*, *Bacillus aureus*, *Staphylococcus aureus*, EP 167, *Pseudomonas aeruginosa*, *Candida albicans* MY 1055, Lactobacilli (*Lactobacillus paraplantarum* LCH 7, *Lactobacillus plantarum* LCH 17, *Lactobacillus fermentum* LPH 1, *Lactobacillus fermentum* CECT 5716, *Lactobacillus brevis* and *Lactobacillus cornyformis* CECT 5711), *Listeria monocytogenes*, *Fusarium culmorum* and *Saccharomyces cervisae* etc.[8]

Cho et al (1998) reported that 4-hydroxybenzoic acid and trans-4-hydroxy cinnamic acid isolated from rice hull have antibacterial activity against most of Gram + ve and some of Gram - ve bacteria at 50% inhibitory concentration of

160 and 100-170 µg/mL respectively and concluded lipophillicity as an important factor that strongly influences the antimicrobial activity of 4-hydroxybenzoic acid as compare to that of trans-4-hydroxy cinnamic acid.[9]

Anthony **(**2009) and Merkl et al (2010) reported that esters of 4-hydroxybenzoic acid (Fig. 2) popularly known as parabens or acid preservatives also exhibit antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Salmonella typhi, Proteus vulgaris, Pseudomonas aeruginosa, Aspergillus niger, Rhizopus nigricans, Chaetomium globosum, Trichophyton intrdigitale, Candida albicans, Salmonella cervisae* due to presence of hydroxyl group. As alkyl chain increases the antimicrobial effect also increases because chain lengthening decreases polarity and also facilitates the compound to cross cell wall of micro-organisms. It is also reported that antimicrobial activity of parabens also increases with hydrophobicity of co-solvent and activity is greatest with most hydrophobic co-solvent (ethanol). [10, 11]

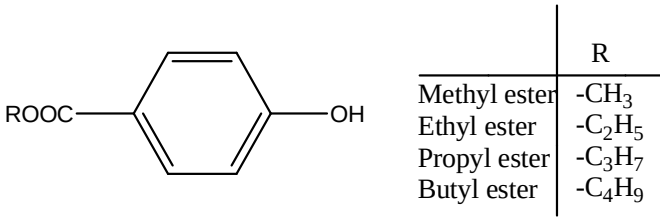


**Figure 2:** Esters of 4-hydroxybenzoic acid (Parabens)

Orsellinic acid (2,4-dihydroxy-6-methylbenzoic acid, Fig.3), a derivative of 4-hydroxybenzoic acid isolated from lichens *Roccella*, *Lecanora*, and *Lobaria yunnanensis*, fungi *Penicillium*, *Hypoxylon*, and *Chaetomium cochliodes* also exhibit antimicrobial activity against a number of micro-organisms as reported by Robbins (2003).[12]

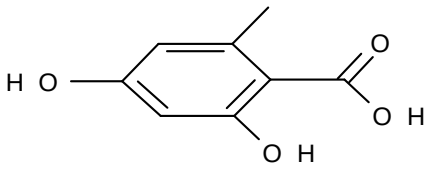


**Figure 3:** Orsellinic acid

A series of phenolic acids like Syringic acid (Fig. 4), Caffeic acid and 4-hydroxybenzoic acid isolated from oil palm root possess in-vitro antimicrobial and fungitoxicity activity against *Ganoderma boninense* with concentration range of 0.5-2.5mg/ml as reported by Chong *et al* (2009).[13]

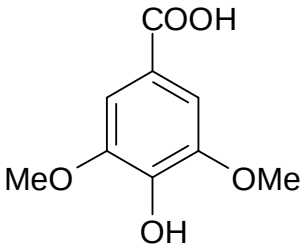


**Figure 4:** Syringic acid

Protocatechuic acid (3, 4-dihydroxybenzoic acid, Fig. 5), a derivative of p-hydroxybenzoic acid which can be synthesized chemically as well as isolated naturally[1] from sources like Spanish heath (*Ericaaustralis*), dog rose (*Rosa canina*), Korean spruce (*Picea koraiensis*), gum-tree (*Eucalyptus grandis*), herb shensi (*Picrorhiza kurrooa*), ferns, buckwheat (*Fagopyrum* spp.), alder (*Alnus* spp.), onion and garlic (*Allium* spp.), Japanese pepper (*Zanthoxylum piperitum*), herb danshen (*Salvia miltiorrhiza*) , sharp-leaf galangal (*Alpinia oxyphylla*), sea buckthorn (*Hippophae rhamnoides*), Japanese honeysuckle (*Lonicera japonica*), mulberry (*Morus alba*), and medlar (*Mespilus germanica*) also exhibit antifungal activity, anti-inflammatory, anti-hepatotoxic[14], anti-oxidant[15], free radical scavenger[16], cytotoxic[17], chemopreventive, apoptotic[18], anti-platelet aggregation, neuroprotective and LDL oxidation inhibitor activity.[1]

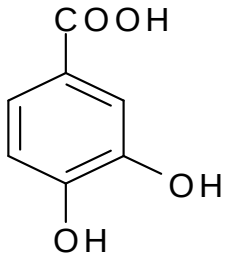


**Figure 5:** Procatechuic acid

**As Antisickling Agent:**

Sickle cell disease (SCD) is a genetic disorder caused due to point mutation in beta-globin gene by replacement of glutamic acid by valine at 6$^{th}$ position of beta-chain of Hb and this mutant Hb or sickle Hb under low oxygen tension polymerizes inside RBC to form gel or fibers that causes drastic increase in red cell deformability. Various efforts have been made to inhibit Hb polymerization to prevent or reduce the crises in SCD.

3,5-dimethoxy-4-hydroxybenzoic acid (Fig.6), another derivative of p-hydroxy benzoic acid inhibit polymerization of sickle Hb which is monitored by UV spectrophotometer procedure using acetic acid by Gamaniel et al, 2000.[19] It also exerts analgesic as well as anti-inflammatory action.

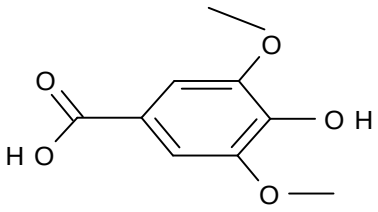


**Figure 6:** 3, 5-dimethoxy-4-hydroxybenzoic acid

Qin et al (2008)[20] reported that vanillic acid (Fig. 7), a derivative of 4-HBA isolated from a number of natural sources such as prickly ash (Fagara spp.), Japnese alder (*Alnus japonica*), spiny oleaster (*Elaeagnus pungens*), Spanish heath (*Erica australis*), Upland cotton (*Gossypium mexicanum*), China berry (*Melica azedarach*), oriental

ginseng (*Panax ginseng*), Korean peroba (*Paratecoma koraiensis*), red sandalwood (*Pterocarpus santalinus*), Dog rose (*Rosa canina*), Shensi (*Picrorhiza kurrooal*), luo shi (*Trachelospermum asiaticum*), ishpingo (*Amburana cearensis*) and egg plant (*Solanum melongena*) also possess anti-sickling activity. Besides this, this derivative also exerts anthelmintic activity, suppress hepatic fibrosis in chronic liver injury[21] inhibit snake venom 5'-nucleotidase[22] and possess protective action to hepatoxicity and nephrotoxicity produced by acetaminophen.

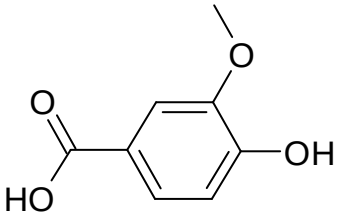


**Figure 7:** Vanillic acid

**As antialgal agent**

Increasing urbinisation and industrilisation accelerated the eutrophication in rivers & seas that lead to harmful algal blooms which in turn causes serious health problems. Medicinal chemists are in race to find effective & safe method to control the development of harmful algae and cynobacterial micro-organisms.

Nakai et.al (2000)[23] isolated ellagic acid, gallic acid (derivative of 4-HBA, Fig 8), pyrogallic acid & (+) catechin from culture of macrophyte *Myriophyllum spicatum* and reported that all isolated phenolic acids inhibited the growth of algae *Microcystis aeruginosa*. Gallic acid has also been reported to inhibit other algae as *Anacystis nidulans* (Cynobacterium) and *Selenestrum capricornutum* (green algae).

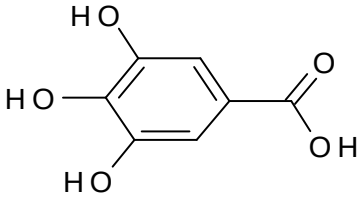


**Figure 8:** Gallic acid

Wang et al (2008)[24] reported that 4-hydroxybenzoic acid and its derivative 3, 4, 5-trihydroxybenzoic acid inhibited the growth of two strains of algae *Microcystis aeruginosa* (toxic FACHB 942 and non- toxic 469). The strain FACHB 942 is found to be more sensitive to 4-hydroxybenzoic acid as compare to non-toxic FACHB 469.

Nakai et al (1996)[25] reported that the position of hydroxyl group and carboxyl group of benzene ring influenced analytical effects of phenolic acids & order of anti-algal effect is

m-hydroxybenzoic acid > o- hydroxybenzoic acid > p-hydroxybenzoic acid

**As antimutagenic agent:**

Birsovaa et al (2005)[26] studied the anticarcinogenic effect of caffeic acid, vanillic acid (Fig. 7), ellagic acid, chlorogenic acid and ferulic acid and found that all these phenolic acid posses anticarcinogenicity by the inhibition of 4-nitrquinoline-1-oxide that in turn induce tongue cancer in rats.

Caffeic acid isolated from a number of natural and agricultural sources as coffee beans, fruits, vegetables, tobacco leaves, olive oils and wine exhibit cytoprotective effect on endothelial cells against oxidized low density lipoprotein and inhibits the oxidation of lipoprotein which is exposed to ferrylmyoglobin and recycles alpha tocopherol from alpha tocopherol radical. Caffeic acid also posses protective effect against the genotoxicity of acridine orange and ofloxacin in *Salmonella typhimurium* as well as in *Euglena gracillis*.[27]

Caffeic acid also inhibit the Trp-P-1 & Glu-P-2 induced mutagenesis by inhibiting the formation of mutagenic & carcinogenic N- nitroso compounds as caffeic acid is the inhibitor of N-nitrosation reaction in vitro.

Derivatives of 4-hydroxybenzoic acid (gallic acid, caffeic acid, vanillic acid, gentisic acid (Fig.9) and syringic acid) possess antimutagenicity against *Salmonella typhimurium* tester strain TA 100 and also against 3-(5-nitro-2-furyl) acrylic acid (5NFAA) and sodium azide induced mutagenicity. Antimutagenicity was expressed as % age of mutagenicity inhibition as per the following formula:

$$\% \text{ mutagenicity} = 100 - [(X1/X2) \times 100]$$

Where X1 = number of revertants per plate in the presence of mutagen & antimutagen

X2 = number of revertants per plate in the absence of antimutagen.

Among these derivatives gallic acid inhibit mutagenesis by 82%.

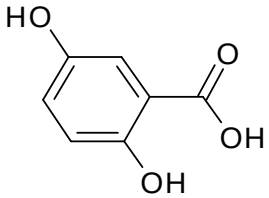


**Figure 9:** Gentisic acid

**As estrogenic agent**

The detection of estrogenic activity of any new compound or substance is very important and especially when it is related to food industry. Ge and Chang (2006)[28]; Lemini et al (1997, 2003)[29-30] reported that ester derivatives of p-hydroxybenzoic acid (parabens) that are structurally similar to 17-beta estradiol exhibited estrogen like property in vivo. Examples of parabens that possess estrogenic activity are methyl paraben, ethyl paraben, propyl paraben, butyl paraben, isopropyl paraben,

isobutyl paraben, 4-n-dodecyl paraben and benzyl paraben.

Among these derivatives butyl paraben possess the most potent and methyl paraben possess the weakest estrogenic effect. These parabens are 1000-1,000,000 times less estrogenic than estradiol, the major estrogenic compound in body.

Darbre (2003)[31] and Okubo (2001)[32] exhibited that estrogenicity of parabens depend on length of their alkyl side chains. Safe concentration of parabens as preservative in cosmetics is 0.1% but this may as high as 0.5%. While using cosmetics parabens penetrate into skin and are quickly absorbed through skin and undergo hepatic metabolism to form 4-hydroxybenzoic acid which is detected in blood and urine of mammals which are under the exposure of parabens[33] though small percentage of parabens may remain as the original form of paraben. Donovan et al (2007) demonstrated that this unchanged original form of paraben is present in human breast tissue & milk.[34]

Byford et al (2002)[35]; Okubo et al (2001)[36] reported that parabens possess estrogen like property in vivo and this estrogenicity is mediated by PR, ER-alpha and PS2 signalling pathway. ER-dependent estrogenic activities of parabens include endocrine system disruption, modification of uterine morphology & physiology in rodents[37-39] reduction of sperm cells, impaired spermatogenesis & reduce cell motility.

Pugazhendhi et al (2007)[40] and Terasaka et al (2006)[41] reported that there is a controversy between parabens exposure and breast cancer development. Pugazhendht & Terasaka noted the altered global patterns of gene expression by estrogen signaling adversely influenced breast cancer development. Darbre et al (2004)[42] reported the presence of parabens in breast tissue of women that on the diagnosis of brest cancer.

Choi and Jeung (2008)[43] reported that Calbindin-D9K (CaBP-9K), a potent biomarker that is presently used for screening or evaluating estrogen like environmental chemicals in vivo and in vitro. is a cytosolic calcium binding protein that is expressed in various mammalian tissues like uterus, placenta, intestine, kidney, pituitary gland and bone. Hong et al, 2004[44], Jeung et al, 1994[45] and Lee et al, 2005[46] reported that CaBP-9K is sensitive to E2 via estrogenic receptor (ER) in uterus & ovary in which ER-alpha & ER-beta are present respectively. The highly induced response to CaBP-9K expression at mRNA & protein level is by isopropyl and butyl paraben. Exposure of parabens increased uterus weight via ER- dependent pathway.

Estrogenic activity of parabens can also be studied by ELISA-based estrogen receptor competitive binding assay.

**As hypoglycemic agent**

Peungvicha et al, 1998[47] discovered that p-hydroxy benzoic acid isolated from roots of Pandanus odorus Ridl (Pandanaceae,) upon oral administration to streptozotocin induced diabetic rats decreases plasma glucose level in dose dependent manner by increasing peripheral glucose consumption. 4-hydroxybenzoic acid increases consumption of glucose in normal as well as in diabetic rat diaphragms but not elevate serum insulin level as well as not alter liver glycogen content in diabetic models.

**As nematicidal agent**

4-HBA and p-coumaric acid isolated from Italian and Algerian Melia azedarach, commonly known as chinaberry, fruits and parts (seeds, wood, and kernels) possess paralytic effect against second stage juveniles of nematode *Meloidogyne incognita*.

**As antiviral agent**

Viruses are the smallest known pathogens that are world widely spread. In present decades almost three quarters of contagious diseases in world are caused by viruses.

Ester derivatives of hydroxybenzoic acid are reported to be widely used for treating infections caused by hepatitis B virus, human papilloma, herpes simplex virus, condyloma acuminate, cervicitis and cervical erosions in human & animals.[48]

Gallic acid, most abundantly present in wines and green tea also reported to possess antiviral effect against HIV-PR (0.8-0.05 microgram) by kinetic analysis, Zhang-Poormans method, a complementary assay as well as by fluorescent probe binding method by using 8-anilino-1 - naphthalene sulfonic acid (ANS) and is also antioxidant, antibacterial, anti-inflammatory, antimutagenic and chemopreventive compound.[49]

3, 4, 5-trimethoxy benzoic acid were used to treat viral infections as hepatitis B, skin mucosa viral infections in human beings. The antiviral activity of a hydroxy benzoic acid ester is higher than that of its corresponding acid. For example, the antiviral activity of propyl gallate is about one time higher than that of gallic acid.

**As antiartherogenic agent**

Ya Quin Ma et al, (2008)[50] reported that various phenolics like p-hydroxybenzoic acid, p-coumaric acid, caffeic acid, sinapic acid, protocatechuic acid and vanillic acid isolated by ultrasonic treatment of Satsuma Mandarian (*Citrus unshiu* Marc.) peels posses antiartherogenic, anti-inflammatory, antiallergenic, antimicrobial, antioxidant, anti thrombotic, cardio protective & vasodilatory effects.

**As teratogenic agent**

Ortho-derivative of 4-hydroxybenzoic acid also reported to exhibit teratogenic activity.

**As anti-inflammatory agent**

Inflammation is characterized by external symptoms as swelling and red color patches on skin that are triggered by a number of mechanisms.[51]

Luecha *et al*., 2009 reported that 4-hydroxybenzoic acid isolated from ethanolic extract of *Vitex glabrata ( Verbenaceae*), commonly known as smooth chaste tree, possess anti-inflammatory effect.[52] V. glabrata is used as food for the treatment of post delivery bleeding, cramps, control foul odour of external genitalia, gastrointestinal disorders as anthelmintic, astringent, stomachic, sexual enhancer and in wound healing. It is also used for treatment of diarrhea, fever & as tonic. Aqueous extract of *V. glabrata* exhibit inhibitory effect to Human Immunodeficiency Virus-1 (HIV-1) reverse transcriptase. Anti-inflammatory activity of PHBA was evaluated by using carrageenan-induced paw edema and cotton pellet induced granuloma formation in rat models and found comparable to diclofenac sodium.

**As Antiplatelet aggregating factor:**

Gallic acid ester (3, 4, 5,-trihydroxybenzoic acid ester) can inhibit the synthesis of thromboxane A2 (TXA2). It has a stronger and faster effect against platelets aggregation than aspirin (ASP) and has been used in solution for injection.

It was also observed that hydroxy benzoic acid esters are more stable than hydroxy benzoic acids viz. propyl gallate was found more stable than gallic acid, especially when it is in weak alkaline condition of the human body (pH 7.4) or intestinal alkaline condition (pH 8.6).

**As antioxidant / preservative agent**

Various physiological and biochemical processes in human body produce oxygen centered free radicals and other reactive oxygen species by products which lead to oxidative damage of biomolecules as lipids, proteins, DNA which eventually lead to a no. of chronic diseases as atherosclerosis, cancer, diabetes, aging and other degenerative disorders in humans.[53]

Merkl et al (2010)[54] reported that phenolic acids and its esters viz. p-hydroxybenzoic acid, gentisic acid, ferulic acid, isoferulic acid, gallic acid, salicylic acid, sinapic acid, coumaric acid, vanillic acid, protocatechuic acid and syringic acid possess good antioxidant effect as reveratol and trolox. Among these compounds gallic acid (3, 4, 5-tri hydroxybenzoic acid) has highest antioxidant effect ($EC_{50}$ 0.0237 micromol/assay).

Pulido et al (2000)[55] demonstrated that antioxidant activity of phenolic acids can be determined by ferric reducing antioxidant power assay (FRAP) whereas Rice-Evans et al. 1996[56], demonstrated that antioxidant activity can also be measured by TEAC (Total equivalent antioxidant capacity) assay.

Hermann et al (1989)[57] reported antioxidant activity of gentisic acid in myeloperoxidase system and also impair the tyrosyl radical catalyzed low density lipoprotein peroxidase.

Beta-resocylic acid (2.4-dihdroxybenzoic acid) having two hydroxy groups bonded in meta position to each other exhibit moderate antioxidant, low DPPH (1,1-Diphenyl 2-picrylhydrazyl radical) and $H_2O_2$ (Hydrogen peroxide) scavenging activity.

Antioxidant activity of the selected phenolic acids alkyl esters (methyl, ethyl, propyl, butyl and hexyl) was investigated by Rancimat method by Cuvelier et al (1992).[58] The esters of 3, 4-dihydroxyphenolic acids (protocatechuic and caffeic acids) exhibited higher antioxidant activities in comparison with the respective phenolic acids. The protection factor of antioxidants was calculated using Rancimat software according to the equation:

PF (%) = IP (oil + antioxidant)/IP (oil) × 100

Where, PF – protection factor

IP – duration of the induction period

**Miscellaneous**

Apart from these activities 4-hydroxybenzoic acid and its derivatives also exert certain other important effects as insecticidal effect, pyrotoxic effect, neurotoxic effect, analgesic effect, anti-allergic effect, cardioprotective effect, vasodilatory effect & hepatotoxic effect.

## CONCLUSION

Looking to the versatile uses of p-hydroxy benzoic acid and its derivatives, possibilities are still there to explore this molecule for more biological activities by further synthesizing its derivatives.

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