Research Article

Evaluation of Biological Activity and Qualitative Analysis of 2, 5-dihydroxybenzoic acid from *Momordica charantia* Fruit

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ABSTRACT

This study aims to reveal the biological activity of 2, 5-dihydroxybenzoic acid from *Momordica charantia* fruit petroleum ether extract. Qualitative phytochemical analysis of these plants confirms the presence of various phytochemicals like sterols, flavonoids, teripenoids, proteins, alkaloids, quinines and anthrocyanins. The PASS Computer program was used in this study to predict the biological activity profile. The results were analyzed to show various biological activities like pharmacological (Gastrointestinal hemorrhage, Antimutagenic, Necrosis, Kidney function stimulant Anti inflammatory, Antipyretic and Glucose oxidase inhibitor). The PASS soft ware is useful for the study of biological activity of secondary metabolites.

Keywords: *Momordica charantia,* 2,5-dihydroxybenzoic acid, biological activity, Anti inflammatory.

INTRODUCTION

According to World Health Organization (WHO), medicinal plants would be the best source to obtain variety of drugs. About 80% of individuals from developed countries use traditional medicines, which has compounds derived from medicinal plants. However, such plants should be investigated to better understand their properties, safety, and efficiency.[1]

Plant products have been part of phytomedicines since time immemorial. This can be derived from barks, leaves, flowers, roots, fruits, seeds.[2] Since ancient times, people have been exploring the nature particularly plants in search of new drugs. This has resulted in the use of large number of medicinal plants with curative properties to treat various diseases. [3]

Medicinal plants contain some organic compounds which provide definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids. [4]

MATERIALS AND METHODS

Preparation of Fruit extracts

Fresh of *Momordica charantia* fruit were collected, washed in water and air dried under shade. Dried fruit were powdered using an electric pulverizer. 10g of the fruit powder was weighed and subjected to extraction with 500 ml of petroleum ether solvent for 8h (60-80°C) using a Soxhlet apparatus.[5,6] The fruit extract thus obtained was concentrated by distillation and dried by evaporation at 40°C.

Qualitative analysis

Preliminary phytochemical analysis

Preliminary screening of the extracts and identification was done by colour tests adapting standard methods. [7]

Test for Alkaloids

Mayer's test

A fraction of extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5 g of potassium iodide in 100 ml of water) and observed for the formation of cream coloured precipitate.

Wagner's test

A fraction of extract was treated with Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml water) and observed for the formation of reddish brown colour precipitate.

Hager's test

A few ml of extract was treated with Hager's reagent (saturated aqueous solution of picric acid) and observed for the formation of prominent yellow precipitate.

Test for Flavonoids

NaoH test

A small amount of extract was treated with aqueous NaOH and HCl, observed for the formation of yellow orange colour.

H_2SO_4 test

A fraction of the extract was treated with concentrated H2SO4 and observed for the formation of orange colour.

Test for Sterols

Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark pink or red colour.

Test for Terpenoids

Liebermann-Burchard test

Extract (1ml) was treated with chloroform, acetic anhydride and drops of H_2SO_4 was added and observed for the formation of dark green colour.

Proteins

Ninhydrin test (Aqueous)

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

Ninhydrin (acetone)

Ninhydrin was dissolved in acetone; the extract was treated with ninhydrin and observed for the formation of purple colour indicating the presence of protein.

Test for Anthraquinones

Borntrager's test

About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia; pink or deep red colourations of aqueous layer indicate the presence of anthraquinone.

The extract was treated with aqueous ninhydrin and observed for the presence of blue colour, indicating the presence of amino acid or purple colour indicating the presence of protein.

Biuret test

The extract was heated in distilled water and filtered. The filtrate is treated with 2% copper sulphate solution, to this added 95% ethanol and potassium hydroxide and observed the formation of pink ethanolic layer indicating the presence of protein.

Test for Phenols

Ferric chloride test

The fraction of extract was treated with 5% ferric chloride and observed for the formation of deep blue or black colour depicts the presence of Phenols

Liebermann's test

The extract was heated with sodium nitrite, added H2SO4 solution diluted with water and excess of dilute NaOH was added and observed for the formation of deep red or green or blue colour.

Test for Quinones

A small amount of extract was treated with concentrated HCl and observed for the formation of yellow colour precipitate shows the presence of quinones.

In Silico Studies

Structure activity analysis

The chemical structure and inhibitory molecules of 2, 5-dihydroxybenzoic acid retrieved from Pub Chem database. The molecules were retrieved in standard 3D SDF format and activity of a compound was predicted using PASS (Prediction of Activity Spectra for substances). The biological activity spectrum of a chemical compound is the set of different types of biological activity that reflect the results of the compound's interaction with various biological entities. Biological activity is defined qualitatively suggesting that the biological activity spectrum represents the "intrinsic" property of a substance depending only on its structure and physical-chemical characteristics. PASS is a software product designed as a tool for evaluating the general biological potential of molecules. PASS provides simultaneous predictions of many types of biological activity based on the structure of compounds. Thus, PASS can be used to estimate the biological activity profiles for virtual molecules, prior to their chemical synthesis and biological testing. If Pa > 0.7 the substance is very likely to exhibit the activity in experiment, but the chance of the substance being the analogue of a known pharmaceutical agent is also high. If 0.5 < Pa<0.7 the substance is likely to exhibit the activity in experiment, but the probability is less, and the substance is unlike known pharmaceutical agent.

RESULTS AND DISCUSSION

Pericarp of *Momordica charantia*

Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites.[8] Analysis of the plant extracts revealed the presence of phytochemicals such as phenols, tannins, flavonoids, saponins, glycosides, steroids, terpenoids, and alkaloids.[9] The results of preliminary phytochemical study of *Momordica charantia* fruit were tabulated in (Table-1). The phytochemical study revealed the presence of flavonoids, alkaloids, sterols, terpenoids, saphonin, proteins and phenols.

Table 1: Phytochemical constituents of the *Momordica charantia* leaf extracts

Chemical tests	*Momordica charantia - Pericarp*
Alkaloid	+
Flavonoids	+
Sterols	+
Terpenoids	+
Tanin	-
Saphonin	+
Anthraquinones	-
Proteins	+
Phenols	+
Quinones	-

+ Detected - Not detected

In silico analysis of phytochemical compounds Structure activity analysis. The three dimensional structure of the secondary metabolites were given as an input for pass server. The pass server provides all the possible activity of the given secondary metabolites (Table-2.)

Table 2: Activities of 2, 5-dihydroxybenzoic acid (Pa – probability of Active, Pi – probability of inactive)

Pa	Pi	Activity	Pa	Pi	Activity
0,962	0,002	Hematemesis	0,795	0,004	(R)-6-hydroxynicotine oxidase inhibitor
0,950	0,002	Ulcer, aphthous	0,796	0,004	Xylan endo-1,3-beta-xylosidase inhibitor
0,944	0,003	Chlordecone reductase inhibitor	0,794	0,004	Tpr proteinase (Porphyromonas gingivalis) inhibitor
0,940	0,002	Benzoate 4-monooxygenase inhibitor	0,792	0,003	2-Oxoglutarate decarboxylase inhibitor
0,940	0,002	Dehydro-L-gulonate decarboxylase inhibitor	0,791	0,002	Diphosphomevalonate decarboxylase inhibitor
0,937	0,003	Antiseptic	0,805	0,017	Necrosis
0,934	0,001	Threonine aldolase inhibitor	0,794	0,007	Lysine 2,3-aminomutase inhibitor
0,932	0,002	Catechol 2,3-dioxygenase inhibitor	0,792	0,005	Mitochondrial processing peptidase inhibitor
0,933	0,002	Glucan endo-1,6-beta-glucosidase inhibitor	0,790	0,003	Bothrolysin inhibitor
0,933	0,002	Gastrointestinal hemorrhage	0,793	0,006	Linoleate diol synthase inhibitor
0,932	0,002	Glutathione thiolesterase inhibitor	0,801	0,014	Fibrillation, atrial
0,931	0,002	Alkane 1-monooxygenase inhibitor	0,790	0,005	Limulus clotting factor B inhibitor
0,929	0,004	Testosterone 17beta-dehydrogenase (NADP+) inhibitor	0,793	0,009	JAK2 expression inhibitor
0,925	0,003	Arylacetonitrilase inhibitor	0,788	0,004	Nephrotic syndrome
0,922	0,005	Acidosis, metabolic	0,794	0,010	Lysase inhibitor
0,915	0,001	Diiodophenylpyruvate reductase inhibitor	0,785	0,002	Vomilenine glucosyltransferase inhibitor
0,915	0,004	Sugar-phosphatase inhibitor			
0,916	0,004	Ubiquinol-cytochrome-c reductase inhibitor	0,788	0,004	Cholestanetriol 26-monooxygenase inhibitor
0,914	0,003	Arylsulfate sulfotransferase inhibitor	0,786	0,003	Uroporphyrinogen-III synthase inhibitor
0,912	0,002	N-hydroxyarylamine O-acetyltransferase inhibitor	0,786	0,003	Aryldialkylphosphatase inhibitor
0,909	0,001	Protocatechuate 3,4-dioxygenase inhibitor	0,785	0,003	Hydroxylamine reductase (NADH) inhibitor
0,911	0,002	NADPH-cytochrome-c2 reductase inhibitor	0,786	0,004	Laccase inhibitor
0,908	0,002	Pectate lyase inhibitor	0,815	0,033	Twitching
0,912	0,006	Aspulvinone dimethylallyltransferase inhibitor	0,785	0,003	6-Pyruvoyltetrahydropterin synthase inhibitor
0,909	0,005	Alkenylglycerophosphocholine hydrolase inhibitor	0,785	0,004	Poly(beta-D-mannuronate) lyase inhibitor
0,904	0,001	4-Hydroxybenzoate 3-monooxygenase inhibitor	0,784	0,003	4-Chlorophenylacetate 3,4-dioxygenase inhibitor
0,904	0,001	Phloroglucinol reductase inhibitor	0,783	0,004	Anthranilate-CoA ligase inhibitor
0,903	0,001	Anthranilate 3-monooxygenase (deaminating) inhibitor	0,781	0,002	3-Hydroxyphenylacetate 6-hydroxylase inhibitor
0,905	0,004	Glucose oxidase inhibitor	0,783	0,005	Hydrogen dehydrogenase inhibitor
0,903	0,002	Alcohol oxidase inhibitor	0,782	0,005	Peptide-N4-(N-acetyl-beta-glucosaminyl)asparagine amidase inhibitor
0,903	0,004	Antiseborrheic	0,780	0,003	6-Carboxyhexanoate-CoA ligase inhibitor
0,898	0,001	Gentisate 1,2-dioxygenase inhibitor	0,780	0,003	Biotin-CoA ligase inhibitor
0,900	0,003	Occult bleeding	0,780	0,003	Homoaconitate hydratase inhibitor
0,900	0,003	Ribulose-phosphate 3-epimerase inhibitor	0,780	0,003	Triacetate-lactonase inhibitor
0,895	0,002	Magnesium-protoporphyrin IX monomethyl ester (oxidative) cyclase inhibitor	0,791	0,014	Protein-glutamate methylesterase inhibitor
0,895	0,002	Corticosteroid side-chain-isomerase inhibitor	0,779	0,002	Glycerol 2-dehydrogenase (NADP+) inhibitor
0,894	0,002	Cis-1,2-dihydro-1,2-dihydroxynaphthalene dehydrogenase inhibitor	0,778	0,003	D-xylulose reductase inhibitor
0,894	0,003	UDP-N-acetylglucosamine 4-epimerase inhibitor	0,779	0,004	Glutarate-semialdehyde dehydrogenase inhibitor
0,895	0,004	Monodehydroascorbate reductase (NADH) inhibitor	0,779	0,004	Nicotinate dehydrogenase inhibitor
0,894	0,003	Glutamyl endopeptidase II inhibitor	0,777	0,003	Salicylate 1-monooxygenase inhibitor
0,896	0,007	Methylenetetrahydrofolate reductase (NADPH) inhibitor	0,776	0,002	3-Hydroxy-4-oxoquinoline 2,4-dioxygenase inhibitor
0,890	0,002	Aspartate-phenylpyruvate transaminase inhibitor	0,777	0,003	N-carbamoyl-L-amino-acid hydrolase inhibitor
0,890	0,003	2-Hydroxyquinoline 8-monooxygenase inhibitor	0,794	0,020	Mucomembranous protector
0,889	0,003	Fatty-acyl-CoA synthase inhibitor	0,774	0,001	Quinate 5-dehydrogenase inhibitor
0,890	0,004	Taurine dehydrogenase inhibitor	0,780	0,008	Gastrointestinal disturbance
0,889	0,002	Peptide alpha-N-acetyltransferase inhibitor	0,773	0,002	Porphobilinogen synthase inhibitor

0,889	0,003	Fibrinolytic	0,775	0,004	Myocarditis
0,896	0,012	Membrane integrity agonist	0,773	0,003	4-Hydroxyglutamate transaminase inhibitor
0,885	0,001	Glycerol dehydratase inhibitor	0,774	0,006	Lipoprotein lipase inhibitor
0,887	0,004	Pullulanase inhibitor	0,771	0,003	Gluconolactonase inhibitor
0,885	0,002	Sulfite reductase inhibitor	0,770	0,002	Succinate-semialdehyde dehydrogenase [NAD(P)+] inhibitor
0,882	0,002	Catechol 1,2-dioxygenase inhibitor	0,770	0,003	Ornithine cyclodeaminase inhibitor
0,884	0,003	Dextranase inhibitor	0,770	0,003	Photoallergy dermatitis
0,881	0,002	3-Hydroxybenzoate 4-monooxygenase inhibitor	0,770	0,003	Mannitol-1-phosphatase inhibitor
0,881	0,003	Gamma-guanidinobutyraldehyde dehydrogenase inhibitor	0,769	0,003	Styrene-oxide isomerase inhibitor
0,891	0,014	CYP2C12 substrate	0,788	0,022	Hepatotoxic
0,877	0,001	2-Pyrocatechuate decarboxylase inhibitor	0,768	0,002	L-3-cyanoalanine synthase inhibitor
0,885	0,009	Shivering	0,770	0,005	Carnitinamidase inhibitor
0,878	0,004	2-Dehydropantoate 2-reductase inhibitor	0,785	0,022	Antieczematic
0,878	0,005	Aldehyde oxidase inhibitor	0,768	0,004	Alkenylglycerophosphoethanolamine hydrolase inhibitor
0,878	0,005	Prolyl aminopeptidase inhibitor	0,766	0,003	Acylphosphatase inhibitor
0,874	0,001	2,6-Dihydroxypyridine 3-monooxygenase inhibitor	0,779	0,015	Mucositis treatment
0,877	0,004	Urinary urge	0,766	0,003	2-Enoate reductase inhibitor
0,876	0,003	Bisphosphoglycerate phosphatase inhibitor	0,766	0,003	Aminocarboxymuconate-semialdehyde decarboxylase inhibitor
0,873	0,002	Aminobutyraldehyde dehydrogenase inhibitor	0,767	0,005	Centromere associated protein inhibitor
0,873	0,003	4-Methoxybenzoate monooxygenase (O-demethylating) inhibitor	0,764	0,003	Nicotine dehydrogenase inhibitor
0,872	0,003	Glucan endo-1,3-beta-D-glucosidase inhibitor	0,764	0,003	Chitosanase inhibitor
0,873	0,005	Feruloyl esterase inhibitor	0,763	0,004	Kidney function stimulant
0,871	0,004	5-O-(4-coumaroyl)-D-quinate 3'-monooxygenase inhibitor	0,783	0,025	CYP2J substrate
0,871	0,004	Allergic contact dermatitis	0,762	0,004	UGT2B1 substrate
0,872	0,004	Allergic dermatitis	0,773	0,015	Membrane permeability inhibitor
0,870	0,004	Arginine 2-monooxygenase inhibitor	0,760	0,003	Creatinine deaminase inhibitor
0,871	0,007	Sphinganine kinase inhibitor	0,761	0,004	Transketolase inhibitor
0,866	0,003	2-Nitropropane dioxygenase inhibitor	0,759	0,003	3-Carboxyethylcatechol 2,3-dioxygenase inhibitor
0,864	0,001	Phenylpyruvate decarboxylase inhibitor	0,770	0,015	Anaphylatoxin receptor antagonist
0,865	0,002	Glyoxylate oxidase inhibitor	0,780	0,025	Acrocylindropepsin inhibitor
0,864	0,003	Pyruvate decarboxylase inhibitor	0,780	0,025	Chymosin inhibitor
0,864	0,003	NADH kinase inhibitor	0,780	0,025	Saccharopepsin inhibitor
0,861	0,003	Sulfite oxidase inhibitor	0,765	0,010	Complement factor D inhibitor
0,858	0,002	Procollagen-lysine 5-dioxygenase inhibitor	0,759	0,004	Leucolysin inhibitor
0,858	0,002	Aryl-alcohol dehydrogenase (NADP+) inhibitor	0,768	0,013	Edema
0,854	0,001	4,5-Dihydroxyphthalate decarboxylase inhibitor	0,757	0,004	Opine dehydrogenase inhibitor
0,855	0,004	Phosphatidylcholine-retinol O-acyltransferase inhibitor	0,756	0,003	D-alanine 2-hydroxymethyltransferase inhibitor
0,854	0,002	Hyponitrite reductase inhibitor	0,754	0,002	Glycerol dehydrogenase (NADP+) inhibitor
0,854	0,003	Butyrate-CoA ligase inhibitor	0,754	0,002	Dopachrome isomerase inhibitor
0,855	0,004	Fusarinine-C ornithinesterase inhibitor	0,754	0,003	Monophenol monooxygenase inhibitor
0,853	0,003	Aryl-acylamidase inhibitor	0,759	0,009	Cyanosis
0,853	0,004	Creatininase inhibitor	0,753	0,003	Camphor 1,2-monooxygenase inhibitor
0,851	0,003	Phosphatidylserine decarboxylase inhibitor	0,750	0,001	4-Hydroxybenzoate 1-hydroxylase inhibitor
0,850	0,004	3-Hydroxybenzoate 6-monooxygenase inhibitor	0,753	0,005	Sarcosine oxidase inhibitor
0,852	0,006	NADPH peroxidase inhibitor	0,760	0,011	Hyperthermic
0,849	0,003	NADPH-ferrihemoprotein reductase inhibitor	0,755	0,008	Lipid metabolism regulator
0,847	0,001	L-ascorbate peroxidase inhibitor	0,753	0,006	Nephritis
0,854	0,009	Postural (orthostatic) hypotension	0,747	0,001	4-Hydroxybenzoate 3-monooxygenase [NAD(P)H] inhibitor
0,849	0,004	Dimethylargininase inhibitor	0,749	0,003	3-Oxoadipate enol-lactonase inhibitor
0,847	0,003	Electron-transferring-flavoprotein dehydrogenase inhibitor	0,746	0,001	4-Hydroxybenzoate decarboxylase inhibitor
0,845	0,002	Oxalate oxidase inhibitor	0,754	0,008	Ototoxicity
0,847	0,004	Antiinfective	0,749	0,003	H+-exporting ATPase inhibitor

0,845	0,003	Methylamine-glutamate N-methyltransferase inhibitor	0,748	0,004	S-formylglutathione hydrolase inhibitor
0,845	0,004	Glutamine-phenylpyruvate transaminase inhibitor	0,750	0,005	Histidine N-acetyltransferase inhibitor
0,845	0,005	Superoxide dismutase inhibitor	0,748	0,004	Thymidylate 5'-phosphatase inhibitor
0,843	0,003	Chenodeoxycholoyltaurine hydrolase inhibitor	0,747	0,003	CDP-4-dehydro-6-deoxyglucose reductase inhibitor
0,843	0,004	Polyamine-transporting ATPase inhibitor	0,746	0,003	Mannan endo-1,6-alpha-mannosidase inhibitor
0,841	0,002	Acidosis, lactic	0,745	0,002	Shikimate 5-dehydrogenase inhibitor
0,843	0,004	2-Hydroxymuconate-semialdehyde hydrolase inhibitor	0,756	0,015	HIF1A expression inhibitor
0,842	0,003	Rhamnulose-1-phosphate aldolase inhibitor	0,745	0,003	Phenylacetate-CoA ligase inhibitor
0,842	0,004	N-benzyloxycarbonylglycine hydrolase inhibitor	0,741	0,002	Glycerol-1-phosphatase inhibitor
0,840	0,003	Crotonoyl-[acyl-carrier-protein] hydratase inhibitor	0,751	0,012	Carboxypeptidase Taq inhibitor
0,840	0,003	Gluconate 5-dehydrogenase inhibitor	0,754	0,015	Dyspnea
0,839	0,002	N-methylhydantoinase (ATP-hydrolysing) inhibitor	0,741	0,003	Glycolate dehydrogenase inhibitor
0,837	0,001	Astringent	0,748	0,010	Optic neuropathy
0,838	0,003	Opheline kinase inhibitor	0,742	0,004	(R)-Pantolactone dehydrogenase (flavin) inhibitor
0,838	0,003	Taurocyamine kinase inhibitor	0,743	0,006	Levanase inhibitor
0,839	0,003	L-glutamate oxidase inhibitor	0,757	0,020	CYP2J2 substrate
0,837	0,003	Long-chain-aldehyde dehydrogenase inhibitor	0,740	0,003	2,3-Dihydroxyindole 2,3-dioxygenase inhibitor
0,837	0,004	Pterin deaminase inhibitor	0,758	0,021	Glycosylphosphatidylinositol phospholipase D inhibitor
0,839	0,006	Nail discoloration	0,743	0,007	Pseudoporphyria
0,837	0,004	Nitrate reductase (cytochrome) inhibitor	0,738	0,003	N-acetyl-gamma-glutamyl-phosphate reductase inhibitor
0,835	0,004	Mucinaminylserine mucinaminidase inhibitor	0,737	0,003	Carboxylesterase inhibitor
0,836	0,007	Urine discoloration	0,737	0,003	N-hydroxy-2-acetamidofluorene reductase inhibitor
0,829	0,001	Orcinol 2-monooxygenase inhibitor	0,737	0,003	Benzaldehyde dehydrogenase (NADP+) inhibitor
0,830	0,003	Nitrite reductase [NAD(P)H] inhibitor	0,753	0,020	Nicotinic alpha6beta3beta4alpha5 receptor antagonist
0,831	0,003	S-alkylcysteine lyase inhibitor	0,736	0,004	Cyclomaltodextrinase inhibitor
0,831	0,004	3-Phytase inhibitor	0,735	0,003	Dimethylmaleate hydratase inhibitor
0,830	0,003	Beta-carotene 15,15'-monooxygenase inhibitor	0,736	0,005	Phosphoinositide 5-phosphatase inhibitor
0,830	0,003	Cyclohexyl-isocyanide hydratase inhibitor	0,734	0,003	Pyruvate dehydrogenase (cytochrome) inhibitor
0,831	0,005	N-acetylneuraminate 7-O(or 9-O)-acetyltransferase inhibitor	0,734	0,003	Trans-2-enoyl-CoA reductase (NAD+) inhibitor
0,831	0,005	Exoribonuclease II inhibitor	0,733	0,004	Inulinase inhibitor
0,829	0,003	tRNA-pseudouridine synthase I inhibitor	0,740	0,011	Hyperuricemia
0,829	0,003	N-Acyl-D-aspartate deacylase inhibitor	0,757	0,029	Gluconate 2-dehydrogenase (acceptor) inhibitor
0,829	0,003	N-acetylneuraminate synthase inhibitor	0,730	0,002	Hippurate hydrolase inhibitor
0,828	0,002	Leukotriene-B4 20-monooxygenase inhibitor	0,730	0,002	Antiinflammatory, intestinal
0,829	0,004	Spermidine dehydrogenase inhibitor	0,730	0,002	Glutamate decarboxylase inhibitor
0,827	0,003	APOA1 expression enhancer	0,732	0,004	Antipyretic
0,827	0,003	N-acylmannosamine kinase inhibitor	0,737	0,010	Optic neuritis
0,826	0,003	Ferredoxin-NAD+ reductase inhibitor	0,729	0,004	Acetylornithine deacetylase inhibitor
0,826	0,003	Naphthalene 1,2-dioxygenase inhibitor	0,734	0,009	Manganese peroxidase inhibitor
0,826	0,003	Histidinol-phosphatase inhibitor	0,728	0,004	2-Haloacid dehalogenase (configuration-inverting) inhibitor
0,827	0,005	Fragilysin inhibitor	0,726	0,003	Myosin ATPase inhibitor
0,832	0,010	Beta-adrenergic receptor kinase inhibitor	0,725	0,003	Trans-pentaprenyltranstransferase inhibitor
0,832	0,010	G-protein-coupled receptor kinase inhibitor	0,732	0,010	Phthalate 4,5-dioxygenase inhibitor
0,825	0,004	Acetylesterase inhibitor	0,724	0,003	Pantoate 4-dehydrogenase inhibitor
0,829	0,008	Prostaglandin-E2 9-reductase inhibitor	0,723	0,003	tRNA nucleotidyltransferase inhibitor
0,824	0,003	1,4-Lactonase inhibitor	0,724	0,003	Aspartyl aminopeptidase inhibitor
0,821	0,001	Tannase inhibitor	0,723	0,003	2,4-Diaminopentanoate dehydrogenase inhibitor
0,824	0,004	Hematuria	0,723	0,003	3-Hydroxybutyryl-CoA dehydrogenase inhibitor
0,823	0,003	Catechol oxidase inhibitor	0,723	0,003	Lysine 6-dehydrogenase inhibitor
0,823	0,004	Amine dehydrogenase inhibitor	0,724	0,004	Tetrahydroxynaphthalene reductase inhibitor
0,820	0,002	Tyrosine-ester sulfotransferase inhibitor	0,724	0,006	Insulysin inhibitor
0,821	0,004	Aldehyde dehydrogenase (pyrroloquinoline-quinone) inhibitor	0,720	0,002	Orotate reductase (NADPH) inhibitor

0,839	0,022	Toxic, respiration	0,721	0,003	Aminomuconate-semialdehyde dehydrogenase inhibitor
0,820	0,004	4-Hydroxyproline epimerase inhibitor	0,721	0,004	Malate dehydrogenase (acceptor) inhibitor
0,820	0,004	Formaldehyde transketolase inhibitor	0,719	0,003	Acetoin dehydrogenase inhibitor
0,817	0,002	Lactaldehyde reductase inhibitor	0,719	0,004	Polygalacturonase inhibitor
0,822	0,007	ADP-thymidine kinase inhibitor	0,717	0,003	Para amino benzoic acid antagonist
0,819	0,004	Irritation	0,738	0,024	Excitability
0,818	0,004	Allyl-alcohol dehydrogenase inhibitor	0,716	0,002	D-amino-acid dehydrogenase inhibitor
0,817	0,004	L-glucuronate reductase inhibitor	0,715	0,002	Prephenate dehydrogenase inhibitor
0,825	0,012	Hepatitis	0,726	0,013	Bronchoconstrictor
0,816	0,003	Tryptophanamidase inhibitor	0,715	0,003	2,4-Dichlorophenol 6-monooxygenase inhibitor
0,816	0,004	Poly(alpha-L-guluronate) lyase inhibitor	0,715	0,004	Cytoprotectant
0,815	0,004	Urethanase inhibitor	0,724	0,013	UDP-glucuronosyltransferase substrate
0,815	0,004	Phospholipid-translocating ATPase inhibitor	0,715	0,004	Glycopeptide alpha-N-acetylgalactosaminidase inhibitor
0,824	0,013	Glutamate-5-semialdehyde dehydrogenase inhibitor	0,716	0,006	D-lactaldehyde dehydrogenase inhibitor
0,825	0,015	Reproductive dysfunction	0,712	0,003	Methylaspartate ammonia-lyase inhibitor
0,814	0,004	Aspartate-ammonia ligase inhibitor	0,712	0,003	Gamma-butyrobetaine dioxygenase inhibitor
0,817	0,008	Non mutagenic, Salmonella	0,712	0,003	Nicotinamidase inhibitor
0,821	0,012	Muscle weakness	0,713	0,004	N-Acyl-D-amino-acid deacylase inhibitor
0,811	0,002	Quinoprotein glucose dehydrogenase inhibitor	0,714	0,005	Phosphatidylglycerophosphatase inhibitor
0,813	0,004	UGT1A6 substrate	0,711	0,004	Protein-Npi-phosphohistidine-sugar phosphotransferase inhibitor
0,813	0,004	Peroxidase inhibitor	0,739	0,031	Ocular toxicity
0,810	0,002	2,5-Dihydroxypyridine 5,6-dioxygenase inhibitor	0,720	0,013	Antiinflammatory
0,811	0,003	Fructan beta-fructosidase inhibitor	0,732	0,025	Stomatitis
0,809	0,001	4-Hydroxybenzoate-CoA ligase inhibitor	0,727	0,021	TP53 expression enhancer
0,828	0,021	Diarrhea	0,709	0,002	(S)-3-hydroxyacid ester dehydrogenase inhibitor
0,823	0,016	Acidosis	0,709	0,003	Penicillin amidase inhibitor
0,809	0,003	Carbon-monoxide dehydrogenase inhibitor	0,709	0,003	4-Coumarate-CoA ligase inhibitor
0,809	0,002	2-Oxoaldehyde dehydrogenase (NADP+) inhibitor	0,711	0,006	Adenomatous polyposis treatment
0,820	0,014	Benzoate-CoA ligase inhibitor	0,706	0,002	Mandelate racemase inhibitor
0,807	0,003	4-Phytase inhibitor	0,715	0,011	Pulmonary edema
0,809	0,005	Withdrawal	0,708	0,005	Interstitial nephritis
0,807	0,004	Preneoplastic conditions treatment	0,708	0,005	Steroid N-acetylglucosaminyltransferase inhibitor
0,819	0,016	Weakness	0,707	0,004	GABA aminotransferase inhibitor
0,806	0,003	Fructose 5-dehydrogenase inhibitor	0,702	0,002	3,4-Dihydroxy-9,10-secoandrosta-1,3,5(10)-triene-9,17-dione 4,5-dioxygenase inhibitor
0,805	0,002	Arylsulfatase inhibitor	0,704	0,004	Indanol dehydrogenase inhibitor
0,806	0,004	Antimutagenic	0,723	0,025	Nephrotoxic
0,805	0,003	Prostaglandin-A1 DELTA-isomerase inhibitor	0,702	0,004	Acaricide
0,805	0,003	P-benzoquinone reductase (NADPH) inhibitor	0,702	0,007	DNA-(apurinic or apyrimidinic site) lyase inhibitor
0,806	0,004	Trans-acenaphthene-1,2-diol dehydrogenase inhibitor	0,749	0,054	Phobic disorders treatment
0,804	0,004	Hypercholesterolemic	0,713	0,019	Protein-disulfide reductase (glutathione) inhibitor
0,804	0,004	4-Nitrophenol 2-monooxygenase inhibitor	0,715	0,025	Anemia
0,812	0,013	Pro-opiomelanocortin converting enzyme inhibitor	0,701	0,014	Muramoyltetrapeptide carboxypeptidase inhibitor
0,807	0,008	Hyperglycemic	0,719	0,033	Drowsiness
0,800	0,002	Vanillyl-alcohol oxidase inhibitor	0,711	0,027	Respiratory failure
0,802	0,004	Phenol O-methyltransferase inhibitor	0,707	0,025	Acylcarnitine hydrolase inhibitor
0,806	0,009	Panic	0,716	0,036	Dermatitis
0,812	0,015	Toxic, vascular	0,712	0,035	Conjunctivitis
0,800	0,004	Arylalkyl acylamidase inhibitor	0,716	0,039	Hematotoxic
0,798	0,002	GABA C receptor agonist	0,706	0,031	Thrombocytopenia
0,798	0,003	4-Hydroxyphenylacetate 3-monooxygenase inhibitor	0,710	0,038	Emetic
0,797	0,003	Mannan endo-1,4-beta-mannosidase inhibitor	0,742	0,015	Coma
0,798	0,003	Glyoxylate reductase inhibitor	0,737	0,011	Oxido reductase inhibitor

CONCLUSION

The three dimensional structure of the secondary metabolites were given as an input for pass server. The pass server provides all the possible activity of the 2, 5-dihydroxybenzoic acid and showed various biological activities like pharmacological Gastrointestinal hemorrhage, Antimutagenic, Necrosis, Kidney function stimulant Anti inflammatory, Antipyretic, Glucose oxidase inhibitor, etc.

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