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RESEARCH ARTICLE

β-Glucuronidase inhibition, estimation of total flavonoid and hydroxyl radical scavenging activity of selected medicinal plants

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ABSTRACT

Various solvent extracts of chosen botanicals were tested for their ability to inhibit β-Glucuronidase. Plant extracts from *Annona squamosa*, *Pergularia daemia*, *Bauhinia racemose*, *Cissus quadrangularis*, and *Albizia lebbeck* were examined in a variety of solvents to check whether they may inhibit β-Glucuronidase. The chloroform extract (IC_{50} = 0.202 mg/ml) of *Pergularia daemia* inhibited the β-Glucuronidase enzyme the most, followed by the chloroform extract (IC_{50} = 0.218 mg/ml) of *Cissus quadrangularis*. Water extract of *Bauhinia racemose* had the lowest activity (IC_{50} = 0.982 mg/ml). Other solvent extracts of chosen plant extract also demonstrated significant efficacy. *Cissus quadrangularis* chloroform extract (IC_{50} = 0.188 mg/ml) has the highest hydroxyl radical scavenging activity. The ethanolic extract (15.25 mg/g) of *Cissus quadrangularis* contained the highest amounts of total flavonoids.

Keywords: *Pergularia daemia*, *Cissus quadrangularis*, β-Glucuronidase, Enzyme inhibition, total flavonoid, arthritis

INTRODUCTION

Many plants have traditionally been utilized in the prevention and treatment of oxidative stress, inflammation, and arthritis. Arthritis is a degenerative disease that has a significant impact on a person's health. Inflammation is a basic protective reaction to living tissue damage and local response. This phenomenon occurs when free radicals, such as reactive oxygen species, interact with biological components, causing cellular damage and tissue destruction. There is growing interest in medicinal plants and traditional plant extract production processes have resulted from an increase in interest in natural healing procedures and the usage of natural products therapeutics [1]. Medicinal plants have several phytochemicals that inhibit the activity of β-Glucuronidase, which is helpful in the treatment of inflammation and arthritic consequences.

METHODOLOGY

Plant material and preparation of plant extracts:
A taxonomist from the Department of Botany, Yeshwant Mahavidyalaya, Nanded-431602, Maharashtra, identified and certified the plants *Annona squamosa*, *Pergularia daemia*, *Bauhinia racemose*, *Cissus quadrangularis*, and *Albizia lebbeck* which were collected from various locations of Nanded district, Maharashtra.

The root bark of the *Annona squamosa*, *Albizia lebbeck*, *Bauhinia racemose*, and the whole plant of *Pergularia daemia*, *Cissus quadrangularis* were obtained and dried in the shadow. The dried plant material and complete plants were crushed into a fine powder using a mixing grinder. The fine powder of the plant was extracted with the Soxhlet apparatus and a range of solvents including water, ethanol, and chloroform. Finally, the filtered extract was concentrated and stored in the refrigerator for future studies.

β-Glucuronidase inhibition:
A 2.5 mM solution of p-nitrophenyl-β-D-glucopyranosiduronic acid was incubated with 5 mg of plant extract in acetate buffer (0.1M, pH 7.4) for 5 minutes, followed by the addition of 0.1 mL of β-glucuronidase (10 g/mL) solution. The mixture was then incubated for another 30 minutes before being stopped with the addition of a 2 mL sodium hydroxide (0.5 N) solution. At 410 nm, the absorbance was measured spectrophotometrically to determine the quantity of reaction product formed. For the comparison investigation, salicylic acid (IC_{50} = 0.113 mg/ml) was employed as the reference drug [2].

Total flavonoids content:
The total flavonoid content of individual plant samples was determined using the aluminum chloride method [3]. The reaction mixture consisted of a 1 ml plant sample, 3 ml methanol, 0.2 ml 10% aluminum chloride, 0.2 ml (1 M) potassium acetate, and 5.6 ml distilled water. After 30 minutes of incubation at room temperature, the absorbance at 415 nm was measured using a UV-VIS spectrophotometer. The standard curve was created using serial dilutions of quercetin (100 mg/ml). The content of flavonoids in diverse plant samples was calculated using the standard curve, and the quantities were represented in quercetin equivalent (mg/g).

Hydroxyl radical scavenging activity:
The previous approach for determining hydroxyl radical scavenging activity was used [4]. 60 µl of 1 mM $Fecl_3$, 90 l of 1 mM 1,10-phenanthroline, 2.4 ml of 0.2 M phosphate buffer (pH 7.8), 150 µl of 0.17 M H_2O_2, and 1.5 ml of varied concentrations of individual plant extract were used in the reaction mixture. A spectrophotometer was used to detect absorbance at 560 nm after the reaction mixture was maintained at room temperature for 5 minutes. α-Tocopherol (IC_{50} = 0.108 mg/ml) was used as a standard compound.

RESULTS

The β-Glucuronidase enzyme inhibition of selected medicinal plants was shown in Table 1. The chloroform extract (IC_{50} = 0.202 mg/ml), ethanol extract (IC_{50} = 0.230 mg/ml) and water extract (IC_{50} = 0.290 mg/ml) of *Pergularia daemia* showed significant β-Glucuronidase enzyme inhibition.

The chloroform extract (IC_{50} = 0.218 mg/ml), ethanol extract (IC_{50} = 0.280 mg/ml) and water extract (IC_{50} = 0.335 mg/ml) of *Cissus quadrangularis* showed maximum β-Glucuronidase enzyme inhibition. The water extract (IC_{50} = 0.552 mg/ml), ethanol extract ((IC_{50} = 0.500 mg/ml) and chloroform extract (IC_{50} = 0.428 mg/ml) of *Annona squamosa* exhibited moderate activity against β-Glucuronidase enzyme. The water extract (IC_{50} = 0.982 mg/ml), ethanol extract (IC_{50} = 0.880 mg/ml) and chloroform extract (IC_{50} = 0.720 mg/ml) of *Bauhinia racemose* showed lowest β-Glucuronidase enzyme inhibitory activity among the selected plants. The chloroform extract (IC_{50} = 0.540 mg/ml), ethanol extract (IC_{50} = 0.502 mg/ml) and water extract (IC_{50} = 0.606 mg/ml) of *Albizia lebbeck* showed moderate β-Glucuronidase enzyme inhibition. The salicylic acid (IC_{50} = 0.113 mg/ml) was used as standard compound for inhibition of β-Glucuronidase enzyme.

The hydroxyl radical scavenging activity of the selected medicinal plants were shown in Table 2.
The highest hydroxyl radical scavenging activity was found in chloroform extract (IC_{50} = 0.188 mg/ml), ethanol extract (IC_{50} = 0.254 mg/ml) and water extract (IC_{50} = 0.271 mg/ml) of *Cissus quadrangularis*. The chloroform extract (IC_{50} = 0.227 mg/ml), ethanol extract (IC_{50} = 0.284 mg/ml) and water extract (IC_{50} = 0.325 mg/ml) of *Pergularia daemia* showed moderate hydroxyl radical scavenging activity = 0.108 mg/ml) was used as standard compound.

Table 1. β-Glucuronidase inhibition by using selected medicinal plants

Sr. No.	Name of plant	Extract	IC_{50} (mg/ml)
1	*Annona squamosa*	(W.)	0.652
		(E.)	0.500
		(Ch.)	0.428
2	*Bauhinia racemose*	(W.)	0.982
		(E.)	0.880
		(Ch.)	0.720
3	*Pergularia daemia*	(W.)	0.290
		(E.)	0.230
		(Ch.)	0.202
4	*Cissus quadrangularis*	(W.)	0.335
		(E.)	0.280
		(Ch.)	0.218
5	*Albizia lebbeck*	(W.)	0.606
		(E.)	0.502
		(Ch.)	0.540
6	Salicylic acid	--	0.113

(W.) – water extract, (E.) – ethanolic extract, (Ch.) – chloroform extract
The results summarized are the mean values of two parallel experiments.

Table 2. Hydroxyl radical scavenging activity of the selected medicinal plants

Sr. No.	Name of plant	Extract	IC_{50} (mg/ml)
1	*Annona squamosa*	(W.)	0.527
		(E.)	0.538
		(Ch.)	0.552
2	*Bauhinia racemose*	(W.)	0.880
		(E.)	0.670
		(Ch.)	0.560
3	*Pergularia daemia*	(W.)	0.325
		(E.)	0.284
		(Ch.)	0.227
4	*Cissus quadrangularis*	(W.)	0.271
		(E.)	0.254
		(Ch.)	0.188
5	*Albizia lebbeck*	(W.)	0.653
		(E.)	0.762
		(Ch.)	0.928
6	α-Tocopherol	--	0.108

(W.) – water extract, (E.) – ethanolic extract, (Ch.) – chloroform extract
The results summarized are the mean values of two parallel experiments.

Table 3. Total flavonoid content of the selected medicinal plants

Sr. No.	Name of plant	Extract	IC_{50} (mg/g)
1	*Annona squamosa*	(W.)	5.5
		(E.)	1.5
		(Ch.)	2
2	*Bauhinia racemose*	(W.)	1.25
		(E.)	2
		(Ch.)	2.75
3	*Pergularia daemia*	(W.)	8
		(E.)	11
		(Ch.)	14
4	*Cissus quadrangularis*	(W.)	9
		(E.)	15.25
		(Ch.)	10
5	*Albizia lebbeck*	(W.)	3.5
		(E.)	2.5
		(Ch.)	1.25

(W.) – water extract, (E.) – ethanolic extract, (Ch.) – chloroform extract
The results summarized are the mean values of two parallel experiments.

The water extract (IC_{50} = 0.527 mg/ml), ethanol extract (IC_{50} = 0.538 mg/ml) and chloroform extract (IC_{50} = 0.552 mg/ml) of *Annona squamosa* showed the considerable hydroxyl radical scavenging activity. The water extract (IC_{50} = 0.880 mg/ml), ethanol extract (IC_{50} = 0.670 mg/ml), and chloroform extract (IC_{50} = 0.560 mg/ml) of *Bauhinia racemose* showed the hydroxyl radical scavenging activity. The lowest hydroxyl radical scavenging activity was found in water extract (IC_{50} = 0.653 mg/ml), ethanol extract (IC_{50} = 0.762 mg/ml) and chloroform extract (IC_{50} = 0.928 mg/ml) of *Albizia lebbeck*. α-Tocopherol (IC_{50}

The total flavonoid content of the selected medicinal plants was shown in Table 3. The highest total flavonoid content was observed in ethanol extract (15.25 mg/g), chloroform extract (10 mg/g), and water extract (9 mg/g) of *Cissus quadrangularis*. The chloroform extract (14 mg/g), ethanol extract (11 mg/g), and water extract (8 mg/g) of *Pergularia daemia* showed a moderate level of total flavonoid content. The water extract (5.5 mg/g), ethanol extract (1.5 mg/g), and chloroform extract (2 mg/g) of *Annona squamosa* showed the lowest content of total flavonoids. The chloroform extract (2.75 mg/g), ethanol extract (2 mg/g), and water extract (1.25 mg/g) of total flavonoid content in *Bauhinia racemose*. The water extract (3.5 mg/g), ethanol extract (2.5 mg/g) and chloroform extract (1.25 mg/g) of total flavonoids are present in *Albizia lebbeck*.

According to certain studies, the presence of many phytochemicals can have a significant inhibitory effect on enzymes. The total flavonoid content of the selected medicinal plants studied is quite high. The plant has a higher concentration of phytoconstituents, which inhibits β-glucuronidase more effectively. Phytochemicals included in the selected medicinal plants trap the relative oxygen species [5]. *Cissus quadrangularis* and *Pergularia daemia* have higher total flavonoid content, which means they have higher β-glucuronidase inhibitory action and hydroxyl radical scavenging ability.

CONCLUSION

According to the studies, *Pergularia daemia* had the highest β-glucuronidase activity, followed by *Cissus quadrangularis* which could be attributable to the presence of greater total flavonoid contents that are easily soluble in the organic solvents. To identify and

purify components from these extracts, more research is needed. This research might be useful in the future to replace the existing pharmaceutical medications.

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