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ANTI MICROBIAL AND ANTI OXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF BARK OF *HEMIDESMUS INDICUS*

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ABSTRACT

Hemidesmus indicus, a plant in the family Apocynaceae, is used in traditional medicine to treat a wide range of conditions. In this study, an ethanolic extract of the plant's bark was used to see if it had any anti-oxidant properties. The antimicrobial activity of the ethanolic extract was tested against the standard ofloxacin using the disc diffusion method. The DPPH method was used to compare the antioxidant activity of the ethanolic extract to that of a standard ascorbic acid. Even in higher amounts, the extract works against ascorbic acid.

KEYWORDS

Hemidesmus indicus, Anti-microbial activity, Anti-oxidant activity and DPPH activity.

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INTRODUCTON

The purpose of this study is to determine the antioxidant and anti-microbal capabilities of *Hemidesmus indicus*. Because it is widely accessible, Indian herbalists utilise Hemidesmus indicus var. indicus (of the Asclepiadaceae family). Some of the conditions it has been used for include antinociceptive, antidiarrheal, renoprotective, antiatherogenic and skin carcinogenic. According to Jain and Singh, Hemidesmus indicus (HI) is used in traditional medicine to alleviate digestive issues. Hemidesmus indicus is a further ulcer therapy option as a result. It seeks to enhance defensive elements in order to attain the typical equilibrium between offensive and defensive factors[1-3]. *Hemidismus Indicus* naturally produces a number of advantageous chemicals that are known for their relaxing and therapeutic properties. The roots, stems, leaves, and flowers of this plant, which has

been the focus of countless scientific studies, have yielded more than a hundred distinct chemicals. Just a few of the numerous substances identified in this plant include hemidesmol, hyperoside, hemidine, hemisine, hexatriconate acid, hemidine, and hemidine. Additionally, it includes 2-hydroxy-4-methoxy benzaldehyde and benzoic acid. Beta-amyrins, betasitosterol, coumarins, delta-dehydro-3-beta-acetate, desmine, glucosides, hemidesmin-1, hemidesmin-2, hemidescine, hemidesmic acid, hemidesmine, hemidesmol, hemidesterol, hemidine, hemisine, hexatriconate acid, hyperoside, indicine, indicusin,

MATERIAL AND METHODS

Drying and size reduction

The plant's bark was meticulously shade dried for fifteen days. They were totally dry for five minutes in a hot air oven with a 45°C temperature setting. Then, they are processed into a fine powder using a mechanical grinder. The extraction procedure was then started using the crushed bark.

Extraction procedure[4]

We extracted 800grammes of air-dried, powdered plant material using a Soxhlet extractor. To create the finished product, leaf powder and ethanol are blended for 24 hours. After that, the dried marc is decocted in water to remove the marc. Distill the solvent, then evaporate the extract until it is completely dry on the water bath.

Preliminary phytochemical analysis[5-8]

Test for Tannins

A little amount of the extract was treated with a solution of ferric chloride at 5% to test for tannins. The presence of green to blue tint was regarded as a sign of tannins.

Lead acetate was used to treat a small quantity of the extract. The presence of a creamy precipitate was regarded as a sign of tannins.

Test for Alkaloids

Mayer's reagent

The extract to be examined is treated with a few drops of diluted 2N HCL and 0.5ml of Mayer's reagent in the first Mayer's Test.

The appearance of alkaloids was confirmed by the white precipitate that was produced.

Wagner's Test

The extract is subjected to 0.5ml of Wagner's reagent and a few drops of 2N HCL. The presence of alkaloids was confirmed by the brown flocculent precipitate that was obtained.

Hager's Test

The extract is subjected to 0.5ml of Hager's reagent and a few drops of diluted 2N HCL. The presence of alkaloids was confirmed by the yellow colour of the precipitate that was produced.

Test for Steroids

Salkowski reaction

Add 2ml each of chloroform and concentrated H2SO4 to 2ml of extract. Firmly shaken, Acid layer exhibits fluorescence that is greenish yellow whereas the chloroform layer appears red.

Liebermann-Burchard reaction

Combine 2ml of extract with chloroform to perform the Liebermann-Burchard reaction. Add 2 drops of concentrated H2SO4 from the test tube's side along with 1-2ml of acetic anhydride.

Libermann reaction

To perform the Libermann reaction, combine 3ml of extract with 3ml of acetic anhydride. Both hot and cold add a few drops of concentrated H2SO4 to get a blue colour.

Tests for Glycosides

Borntrager's test

A 50mg extract was hydrolyzed for two hours in a water bath with 2ml of strong HCl, and then it was filtered. 3ml of CHCl3 was added and mixed with 2ml of filtrate hydrolysate. After separating the CHCl3 layer, a 10% NH3 solution was added. Anthraquinone glycosides are present when a pink colour forms.

Baljet test

The sodium picrate-treated alcoholic or aqueous extract test solution is used in the Baljet test. Glycosides are present when a yellow to orange colour is present.

The Keller-Kiliani test

Involves treating 2ml of the test solution with a few drops of ferric chloride solution, mixing it, and then adding ferric chloride solution containing sulphuric acid. This creates two layers. Glycosides are present because the lower layer is reddish brown and the higher layer is blue green.

Saponins test

A tiny amount of dry extract was cooked with water and then allowed to cool for the foam test. After that, it was shaken ferociously for a minute. It was thought that the presence of persistent, honey comb-like foam indicated the presence of saponins.

Test for Saponins

Foam's test

A small amount of dry extract was boiled with water and allowed to cool. It was then shaken vigorously for a minute. The formation of persistent honey comb like froth was considered as a positive test for saponins.

Test for Sugars

The Molisch's test

It was used to determine whether carbs were present. The extract was combined with 1ml of an alcoholic solution of -napthol at a 10% concentration. A violet ring that formed at the junction after 1ml of strong sulfuric acid was carefully poured along the test tube's sides is considered a positive sign that carbs are present.

Fehling's test

Fehling's solution A and B were heated in equal parts with 5ml of extract solution. The presence of reducing sugars is confirmed by the colour transition from blue to green to reddish orange.

The Benedict's test

Which involved heating 5ml of the extract's solution with 5ml of Benedict's reagent.

A positive test for reducing sugars was seen as a green, yellow, or orange-red precipitate.

Test for Proteins

Biuret test

A small portion of extract was treated with Biuret reagent.

Xanthoprotein test

Mix 3ml. T.S. with 1ml.conc. H_2SO_4. White precipitate is formed. Boil. Solution turns black or brownish due to Lead sulphide formation.

Biological evolution

Anti-microbial activity[9-14]

Test Organisms Bacterial strains were obtained from National Chemical Laboratories (NCL), Pune and Microbial Type Culture Collection (MTCC), Chandigarh. The strains used for the present study were Staphycococcus aureus (NCIM 2079), Bacillus subtilis (NCIM 2063), Escherichia coli (NCIM 2931, Proteus vulgaris (NCIM 2027).

Procedure

Researchers applied the disc diffusion technique to ascertain the extract's antibacterial capabilities. The nutritional agar media was sterilised using an autoclave. They were applied on petridishes and allowed to dry to a consistent depth of 4mm at room temperature in an aseptic chamber. Escherichia coli, Staphylococcus aureus, and Proteus vulgaris were used as test organisms in antibacterial research, and sterile swabs containing bacteria were used to disperse them over the media. The residual ethanol extract was diluted with DMSO to a concentration of 100, 250 and 500g/disc before being utilised in the experiment. The ideal dosage was 5g/disc of ofloxacin. Finally, to confirm that the zone of inhibition did not overlap, six-millimeter-thick, sterile filter paper discs were put over the solidified agar and immersed in a specific concentration of plant extracts. The substance on these plates took 30 minutes to diffuse into the agar substrate. The creature was cultured in petridishes for 24 hours at 37 degrees Celsius. After the incubation period, the zone of inhibition produced by the samples and the standard was evaluated. Three different runs of each test were completed.

Antioxidant activity by DPPH method[15-20]

In vitro testing of the isolated compound's antioxidant abilities involved reducing the production of DPPH free radicals. Methods differ substantially in terms of the created radical, the reproducibility of the creation process, and how the end point is established. The DPPH solution was created by weighing out 22mg of DPPH and then dissolving it in 100ml of ethanol. To create 100M DPPH solutions from 18 millilitres of this stock solution, 100 microliters of the stock solution were diluted. The standard solution was made by accurately weighing 10.5mg of ascorbic acid and dissolving it in the same amount of DMSO to produce 10.5mg/ml. The sample solution was made by accurately weighing each compound and dissolving it in one ml of freshly distilled DMSO to yield solutions with a concentration of 2.1mg/ml.

To create a varied concentration, the extract was mixed with an ethanolic solution of the DPPH radical. The reaction mixture was completely

vortexed and left to rest for 30 minutes at room temperature. At a wavelength of 517nm, the mixture's absorbance was compared to that of a control solution. The ascorbic acid solutions and sample concentration are both 100g/ml. Calculating the percentage of DPPH radical scavengers inhibited required the use of the following formula:

DPPH radical scavenging activity (%) = $\frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100$

Abs sample was the absorbance of DPPH radical and sample/standard, whereas Abs control was the absorbance of DPPH radical and ethanol.

The scavenging activity was measured in terms of IC50, or the sample concentration needed to produce a 50% drop in the DPPH radical signal's strength. The results were done at least in triplicate.

RESULTS AND DISCUSSION

Discussion

According to the results of a Phytochemical Evolution study, the ethanolic extract of the plant is less active than the standard. The extracts were diluted using concentrations ranging from 100g/ml to 500g/ml. If you want to get the most out of the DPPH reagent method, you'll have to use an extract with 500g/ml of concentration. There is a corresponding percentage of inhibition for each concentration in g/ml at 50, 100, 150, 200. The standard ascorbic acid was compared to these results.

Table No.1: Phytochemical constituents present in total ethanolic extract and fractions of *Hemidesmus indicus* and *Ficus religiosa*

S.No	Tests	*Hemidesmus indicus*
1	Tannins	+
2	Alkaloids	-
3	Steroids	+
4	Glycosides Cardiac	+
	Anthraquinone	-
	Saponin	-
	Flavanoids	+
	Coumarins	-
5	Sugars	+
6	Protiens	+

(+) - Indicates the presence of the phyto constituent

(-) - Indicates the absence of the phyto constituent

Antimicrobial activity

S.No		Alcoholic extract of *Coriandrum Sativum*				Ofloxacin		
		Zone of inhibition in mm						
			100µg/ml	250mg/ml	500µg/ml	100µg/ml	250mg/ml	500µg/ml
1	Name of the organisms	*Staphylococcus aureus*	9	13	21	11	16	24
		Bacillus subtilis	7	14	18	12	16	26
		Escherichia coli	6	14	21	13	17	28
		Proteus vulgaris	10	16	22	11	15	24
2	Control	DMSO	-	-	-	-	-	-

Anti-oxidant activity

S.No	Compound	Concentration	% inhibition
1	Ethanolic extract	50	50
		100	62
		150	74
		200	85
2	Standard (Ascorbic acid)	50	60
		100	75
		150	80
		200	95

CONCLUSION

Extract made from the powdered bark of plant Hemidesmus indicus, was found to have significant anti-microbial anti-oxidant activity in the current study. There is a good anti-microbial and anti-oxidant activity in the plant extract, but there is a lack of activity in the extract. The activity of this compound has been comparable to that of the standard drug.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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