Research Article

Antibacterial and Antioxidant, Anti-Inflammatory Study of Leaves and Root *Delonix regia*

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ABSTRACT
Carbon tetrachloride, chloroform, ethanolic and hexane extracts of the root and leaves of *Delonix regia* were evaluated separately for antimicrobial and antipyretic activities. All the extracts were tested against certain Gram positive and Gram negative organisms by well diffusion method. In the methodology, antimicrobial activity was observed for extracts against all the tested organisms. Anti-inflammatory activity of ethanolic and chloroform extract are maximum as well as antioxidant activity is maximum for ethanolic extract studied by DPPH Method.

Keywords: *Delonix regia,* antimicrobial activity, antipyretic activity metahnolic extract.

INTRODUCTION

Plant delonix regia belong to family fabacae is a plant occur in all over Asia. This plant occurs all over the world having the following characteristics: Height 35-40 feet with regular outline like crown having fast growth rate, leaf arrangement is alternate and bippinate type with entire margine. Leaf shape is oblong and ev-ergreen type of leaf. Flowers are orange red in color and very showy. Fruits are elongated pod like about 12inches or more. Initially fruit covering is green is be-come dry, hard and turns brown. Bark of tree is verti-cal thick nothrons uniform. Root of this tree is not much deep with paste resistance. To validate antiseptic property of plant material. The plant material is extracted and studied for antibacterial and antifungal activity.

Preparation of Extracts

Preparation of plant extracts

Plant material collected wash with water, shade dried ,weight of plant material is recorded and material was shade dried for 8 days and then powdered in pulverized ,powder is used for further study**.**

Preparation of various extract of *Delonix regia*

dry stem of the plant collected from western ghat region Maharashtra. Dried stems are cut into small pieces these pieces are then grinded. The grinded sample is green brown in color with a special smell.

Preparation of carbon tetrachloride extract

This powder stirred in non-polar solvent such as CCl_4, for 1/2 hour then it is refluxed for 1/2 hour this is performed for extraction of non-polar component from powder. After extraction the CCl_4 layer is distilled to recover solvent and to get a brown colored liquid fraction which shows single spot on thin layer chromatography.

Preparation of chloroform extract

The residue of CCl_4 extraction is used for further study. This residue is mixed with $CHCl_3$ and stirred for 1/2 hour and then refluxed for 1 hour. After filtration the filtrate is distilled to get $CHCl_3$ Fraction which is Red-brown colored liquid.

Preparation of ethyl acetate extract

Then the Residue of $CHCl_3$ is used for extraction with Ethyl acetate stirred well & refluxed for 1 hour then filtered. Filtrate is then distilled and fraction of Ethyl acetate is collected it shows no spot on TLC plate. Conclusion is that no organic compound is present.

Preparation of methanol extract

The Ethyl acetate residue is further mixed with methanol & stirred for 1/2 hr & refluxed for 1hr. Then it is filtered & filtrate is distilled out.Methanol fraction is yellow brown in colour and show single spot On TLC platte. The remaining residue also have smell & it is observed that residue is insect repellant.

Table 1: Color, consistency and percentage of yield of various extracts of *A. cadamba* root

S. No	Solvent	Color	Consistency	Yield (%) w/w
1	Petroleum ether	Pale green	Waxy	1.78
2	Chloroform	Dark Yellow	Greasy	2.54
3	Methanol	Deep green	Greasy	6.4
4	Aqueous	Pale brown	Sticky	

Microorganisms

G (+) Staphylococcus epidermidis Staphylococcus oureus Bacillus paludis Bacillus subtilis.

G (-) Escherichia Coli ,Pseudomonus aeruginosa, Shigella flaxinely ,Enterobacter aero genes.

These organisms were identified a procured from Nikhil analytical laboratory Sangli, Maharashtra.

Antimicrobial Activity:

The agar diffusion method [11] was used to evaluate the antimicrobial activity. Bacteria were cultured overnight at 37 ^{o} C in Mueller Hinton 10 µl Broth (MHB, Oxoid) and fungi at 28 ^{o} C for 72h in Potato Dextrose Broth (PDB, Oxide) and used as inoculums. A final inoculums, using 100 µl of suspension containing 10 ^{8} CFV/ml of bacteria 10 ^{4} spore/ml of fungi spread on Mueller Hinton Agar (MHA) and Potato Dextrose Agar (PDA) medium respectively.

The disc (6 mm in diameter) was impregnated with 10 µl of 75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml of each extracts and for each organism placed on seeded agar. Ciprofloxacin and Fluconazole (75 µl/ml, 50 µl/ml, 25 µl/ml, 10 µl/ml and 5 µl/ml) were used as positive control bacteria and fungi respectively. The test plates were incubated at 37 ^{o} C for 24h for bacteria and at 28 ^{o} C for 72h for fungi depending on the incubation time required for a visible growth

Study of anti – inflammatory activity (In – vitro models)

Cassia fistula leaves extract was screened for anti – inflammatory activity by using inhibition of albumin denaturation technique which was studied according to Muzushima and Kabayashi with slight modification at the doses of 200 mg/kg. The standard drug and test compounds were dissolved in minimum quantity of DMF and diluted with phosphate buffer (0.2 M, pH 7.4). Final

concentration of DMF in all solutions was less than 2.5%. Test solution (1 ml) containing different concentrations of drug was mixed with 1ml of 1mM albumin solution in phosphate buffer and incubated at 27 ºc + 1 ºc in water bath for 10 min. After cooling, the turbidity was measured at 660 nm (UV – Visible Spectrophotometer SL – 159, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and average is taken

Statistical Analysis

The percentage inhibition of denaturation was calculated by using following formula.

% of Inhibition = 100 X [Vt / Vc – 1]

Where,

Vt = Mean absorbance of test sample

Vc = Mean absorbance of control

DPPH scavenging test

Quantitative measurement of radical scavenging properties was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (or 80% MeOH as blank) and 5 mL of a 0.004% (w/v) solution of DPPH in methanol. Different known antioxidants, vitamin E, and butylatedhydroxytoluene (BHT, Sigma) were used for comparison or

as a positive control. Discoloration was measured at 517 nm after incubation for 30 min. Measurements was taken at least in triplicate. DPPH radical's concentration was calculated using the following equation:

DPPH scavenging effect (%) = Ao – A1 / Ao X100

Where Ao was the absorbance of the control and

A1 was theAbsorbance in the presence of the sample

The actual decrease in absorption induced

by the test compounds was compared with the positive controls. The mean OD 517 results of DPPH scavenging activity was recorded

Table 1: Antibacterial activity of methanolic extract from extract from leaves of *Delonix regia*

Bacterial	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)	In mm	In mm	In mm	In mm	In mm
1. Staphylococcus epidermidis	05	03	09	10	9
2. Staphylococcus oureus	06	04	10	8	8
3. Bacillus paludis	04	02	11	10	8
4. Bacillus subtilis	04	02	11	10	7
G (-)					
1. Escherichia Coli	05	03	7	6.5	5.5
2. Pseudomonus aeruginosa	05	03	6	7	5.5
3. Shigella flaxinely	06	04	5.5	7.5	8
4. Enterobacter aero genes	06	04	5	3	2.

Table II: antifungal activity of ethanilic extract from leaves of *Delonix regia*

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
1. Candida albicans	3	5	7	5	7
2. Aspergillus fumigatus	2	4	5	9	4
3. Aspergillus niger	4	03	12	8	9

Anti-inflammatory activity of cassia fistula leaves (ethanol extract)

In-Vitro Anti – inflammatory activity of cassia fistula	Dose (mg / kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	5ml / kg	0.098	----
Standard (Ibuprofen)	100mg/kg	0.182	85.71
Petroleum ether extract	200mg/kg	0.151	54.08
Chloroform extract	200mg/kg	0.141	43.87
Ethyl acetate extract	200mg/kg	0.124	26.53
n-Butanol	200mg/kg	0.167	70.40
Ethanol	200mg/kg	0.175	72.40

Anti inflammatory activity of *Delonix regia* (ethanol extract)

In-Vitro Anti – inflammatory activity of cassia fistula	Dose (mg / kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	5ml / kg	0.098	----
Standard (Ibuprofen)	100mg/kg	0.188	
Petroleum ether extract	200mg/kg	0.153	22.87
Chloroform extract	200mg/kg	0.147	22.77
Ethyl acetate extract	200mg/kg	0.121	55.33
n-Butanol	200mg/kg	0.177	6,21
Ethanol	200mg/kg	0.185	1.65

Result Table I: Antioxidant activity of leaves *Delonix regia*

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	12.11	09.53	08.47
0.1	46.91	11.64	12.53	09
0.2	49.24	10.24	12.50	11
0.3	57.57	12.12	10.00	12

DISCUSSION

The results of Antimicrobial activity were done for all the five, pet ether, chloroform, acetone, and ethanol and aqueous extracts. During antimicrobial study ethanol extracts showed maximum zone of inhibition against almost all organisms in cup plate method.Ehanolic extract show highest activity.

Antiinflametry activity of delonix regia is very good as compared with standards There is a strong need for effective antioxidants from natural sources as alternatives to synthetic antioxidant in order to prevent the free radicals implicated diseases which can have serious effects on the cardiovascular system, either through lipid per oxidation or vasoconstriction. The extracts and essential oils of many plants have been investigated for their antioxidant activity[5-7]. Secondary metabolites such as polyphenols are not required for plant development and growth, but are involved in plant communication and defense[8-9]. Polyphenols interact with pathogens, herbivores, and other plants; they protect from ultraviolet radiation and oxidants, repel or poison predators and attract beneficial insects or microbes[10-11]. Therefore, in this study, the antioxidant properties of the methanol extracts of leaves and stems of plant like of re examined for DPPH radical scavenging activity according to the method described and the results of the screening are shown in table 4and table 5 as comparable with known antioxidant BHT. In terms of antioxidant activity, all the extracts investigated exhibited a rather good .In particular, leaves (ethanol extract) of ***Delonix regia*** displayed the highest activities as antioxidant activity as removal of the stable radical DPPH and the lowest activity were found in CCl4 extract of bark. As expected, the overall activity of the raw extracts was lower than that of commercial antioxidant BHT, the reference antioxidant.

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