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ANTIBACTERIAL AND ANTIOXIDANT, ANTI-INFLAMMATORY STUDY OF LEAVES AND BARK OF *CASSIA FISTULA*

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ABSTRACT

Ethanol, methanol, chloroform and carbon tetrachloride, and hexane extracts from Cassia fistula were investigated for their in vitro antimicrobial properties, along with anti-inflammatory activity. All three extract of Cassia fistula were different in terms of their antibacterial activities. The Ethanol extract showed a stronger and broader spectrum of antibacterial activity. study was also carried out to evaluate the in-vitro antioxidant activities of ethanol, chloroform and carbon tetrachloride extract of Cassia fistula s .This was achieved by screening the two plant extracts at varying concentrations (10-50g/ml)using DPPH radical scavenging activity, reducing power assay, hydroxyl radical scavenging activity and nitric oxide radical scavenging activity. The results were analyzed statistically which showed that ethanol extract Cassia fistula had more antioxidant activity than standard antioxidant.Al extract was also studied for their anti-inflametry activity.

Keywords: Leaves, Bark, Antibacterial, Antioxidant, Cassia fistula, DPPH and Anti-inflammatory

INTRODUCTION

Cassia fistula, known as the golden shower tree and other names, is a flowering plant in the family Fabaceae, native to southern Asia, from southern Pakistan east through India to Myanmar and south to Sri Lanka. It is the national tree of Thailand, and its flower is Thailand's national flower. It is also state flower of Kerala in India and of immense importance amongst Malayali population. It is a popular ornamental plant and is an herbal medicine. The golden shower tree is a medium-sized tree, growing to 10–20 m (33–66 ft) tall with fast growth. The leaves are deciduous, 15–60 cm (6–24 in) long, pinnate with 3–8 pairs of leaflets, each leaflet 7–21 cm (3–8 inches) long and 4–9 cm (1.5–3.5 in) broad. The flowers are produced in pendulous racemes 20–40 cm (8–15 in) long, each flower 4–7 cm diameter with five yellow petals of equal size and shape. The fruit is a legume, 30–60 cm (12–23 in) long and 1.5–2.5 cm (0.5–1 in) broad, with a pungent odor and containing several seedsCassia fistula is widely grown as an ornamental plant in tropical and subtropical areas. It blooms in late spring. Flowering is profuse, with trees being covered with yellow flowers, many times with almost no leaf being seen. It will grow well in dry climates. Growth for this tree is best in full sun on well-drained soil; it is relatively drought tolerant and slightly salt tolerant. It will tolerate light brief frost, but can get damaged if frost persists. It can be subject to mildew or leaf spot, especially during the second half of the growing season. The tree will bloom better where there is pronounced difference between summer and winter temperatures.

Cassia fistula linn (Caesalpinaceae) tree is one of the most widespread in the forests of India, usually occurring in deciduous forests. The whole plant possesses medicinal properties useful in the treatment

of skin diseases, inflammatory diseases. Damage to cells caused by free radicals is believed to play a central role in the aging process and in disease progression. Antioxidants are our first line of defense against free radical damage, and are critical for maintaining optimum health and wellbeing. The need for antioxidants becomes even more critical with increased exposure to free radicals. Pollution, cigarette smoke, drugs, illness, stress, and even exercise can increase free radical exposure. Because so many factors can contribute to oxidative stress, individual assessment of susceptibility becomes important. Many experts believe that the Recommended Dietary Allowance (RDA) for specific antioxidants may be inadequate and, in some instances, the need may be several times the RDA.

MATERIAL AND METHOD

Plant Material: The plant materials used in this study, Cassia fistularoots, seed and leaves of were collected from the field in Khandala Tal. Shrirampur, Dist Ahmednagar identified by Dr. A. K. Mohite, R.B.N.B College, Shrirampur, Maharashtra, India. A voucher specimen of the collected sample was deposited in our institutional herbarium for the reference.

Preparation of various extract of Cassia fistula: In present study we use dry stem of the plant is collected from Khandala Tal. Shrirampur Maharashtra. Dried leaves and bark pieces are cut into small pieces these pieces are then grinded. The grinded sample is used for study. This powder stirred in non-polar solvent such as CCl_4, for 1/2 hour and 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. The residue of CCl_4 extraction is used for further study. This residue is mixed with $CHCl_3$ & stirred for 1/2 hour and then refluxed for 1 hour. After filtration the filtrate is distilled to get $CHCl_3$ Fraction which is green colored liquid.

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 & fraction of Ethyl acetate is collected it shows no spot on TLC plate. Conclusion is that no organic compound is present. 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 & show single spot On TLC plate. The remaining residue also have smell & it is observed that residue is insect repellant and used as fertilizer.

DPPH Scavenging Test: Quantitative measurement of radical scavenging property 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] ×100; Where Ao was the absorbance of the control and A1 was the absorbance 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 were recorded.

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. MIC values were also studied for microorganisms by turbidimetric method, which were determined as sensitive to the extracts in cup plate method. MIC was defined as the lowest concentration of extract that inhibit 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

RESULTS AND DISCUSSION

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 1 & table 2 as comparable with known antioxidant BHT. In terms of antioxidant activity, all the extracts investigated exhibited a rather high degree of activity (more than 40%). In particular, leaves (ethanol extract) of Cassia fistula 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.

The results of Antimicrobial activity were done for all the five, pet ether, chloroform, acetone, and methanol and aqueous extracts. During antimicrobial study methanolic extracts showed maximum zone of inhibition against almost all organisms in cup plate method. The methanolic extract from roots of Cassia fistula showed a good inhibition against all the bacterial Strains tested (MIC between 10-80 µg/ml). The gram (+) bacteria were sensitive with gram (-) bacteria and some common fungi.

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Table 1: Antibacterial activity of methanolic extract from extract from leaves of *cassia fistula*

Bacterial	Extract ethanolic (mm)	Extract CCl_4 (mm)	Cefotax (mm)	Penicil (mm)	Tetrax (mm)
G (+)					
1. Staphylococcus Epidermidis	14	8	7	10	9
2. Staphylococcus Oureus	11	12	10	8	8
3. Bacillus paludis	12	8	11	10	8
4. Bacillus subtilis	14	8	11	10	7
G (-)					
1. Escherichia Coli	8	8	7	6.5	5.5
2. Pseudomonus aeruginosa	8	9	6	7	5.5
3. Shigella flaxinely	8	8	5.5	7.5	8
4. Enterobacter aero genes	9	5	5	3	2.

Table 2: antifungal activity of ethanilic extract from leaves of cassia fistula

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
Candida albicans	5	7	7	5	7
Aspergillus Fumigatus	6	9	5	9	4
Aspergillus niger	5	14	12	8	9

Table 3: Antibacterial activity of methanolic extract from bark of cassia fistula

Bacteria	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
G (+)					
Staphylococcus epidermidis	12	11	13	12	12
Staphylococcus oureus	12	10	10	8	9
Bacillus paludis	10	9	10	11	7
Bacillus subtilis	14	9	12	11	6
G (-)					
Escherichia Coli	8	12	7	5.5	6.5
Pseudomonus aeruginosa	10	12	8	8	7.5
Shigella flaxinely	11	7	5.5	4.5	6
Enterobacter aero genes	15	8	5	3	2.

Table 4: Antibacterial activity of methanolic extract from bark of cassia fistula

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
Candida albicans	12	7	6	6	8
Aspergillus fumigatus	9	12	8	6	9
Aspergillus niger	12	11	9	9	5

Table 5: Antioxidant activity of leaves

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	36.6O	26.53	46.47
0.1	46.91	26.64	22.53	50
0.2	49.24	48.24	38.50	54
0.3	57.57	54.12	40.00	30

Table 6: Antioxidant activity of bark

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_3$	CCl_4
0.05	45.1	32	25	23
0.1	46.91	27	23	32
0.2	49.24	29	21	23
0.3	57.57	32	20	28

Table 7: 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

Table 8: Anti inflammatory activity of cassia fistula bark (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	86.71
Petroleum ether extract	200mg/kg	0.153	52.08
Chloroform extract	200mg/kg	0.147	41.77
Ethyl acetate extract	200mg/kg	0.121	25.33
n-Butanol	200mg/kg	0.177	74.40
Ethanol	200mg/kg	0.185	78.40

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