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Research Article

ANTIBACTERIAL, ANTIOXIDANT AND ANTI-INFLAMMATORY STUDIES OF LEAVES AND ROOTS OF *SOLANUM XANTHOCARPUM*

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ABSTRACT

Carbon tetrachloride, chloroform, ethanolic and hexane extracts of the root and leaves of *Solanum xanthocarpum* 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: *Solanum Xanthocarpum,* Antimicrobial Activity, Antipyretic Activity Methanolic Extract.

INTRODUCTION

Yellow Berried Nightshade is an extremely vital herb in Hindu medicinal practice. Revered for its therapeutic properties, the herb is found in many parts of India[1-5].

Yellow Berried Nightshade is one of the chief ingredients in *Dashamoola Rasayanam*, an Ayurvedic preparation for the treatment of respiratory ailments. The herb is also a digestive and a carminative, which facilitates the treatment of gastrointestinal problems. The fruit and inflorescences are reportedly edible to humans[6-10]. The fresh leaves are fed to cattle.

The fragrant orange flowers attract pollinators. It is Sapwood white with a light yellow tinge becoming creamy yellow on exposure and is not clearly differentiated from the heartwood is stated to be one of the most frequently planted trees in the tropics[11-16]. A yellow dye is obtained from the root bark. PLANT flowers are an important raw material in the production of 'attar', which is Indian perfume with sandalwood (Santalum spp.) base in which one of the essences is absorbed through hydro-distillation. The flowers exhibit slight anti-implantation activity in test animals. PLANT extracts exhibit nematicidal effects on Meloidogyne incognita. The dried bark is used to relieve fever and as a tonic. An extract of the leaves serves as a mouth gargle[17-23].In present study Leaves and bark are studied for antioxidant and anti-inflammatory activity.

MATERIALS AND METHODS

Intact roots, collected carefully from experimental plants inhabiting in forests of Sahyadri region in Maharashtra district, Ahmed Nagar during June 5010, by excavating adjacent soil without causing damage to outer root profile .Plant was identified by Dr. A.K. Mohite, Head, Department of Chemistry, R.B.N.B College Shrirampur dist Ahmednagar Maharashtra photocopy of plant material is preserved .

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 *Solanum xanthocarpum*

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 1/5 hour then it is refluxed for 1/5 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 $CHCl_4$ and stirred for 1/5 hour and then refluxed for 1 hour. After filtration the filtrate is distilled to get $CHCl_4$ Fraction which is Red-brown colored liquid.

Preparation of ethyl acetate extract

Then the Residue of $CHCl_4$ 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 1/5 hr & refluxed for 1hr. Then it is filtered & filtrate is distilled out. Methanol fraction is yellow brown in color and show single spot On TLC plate 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.

Microorganisms: G (+) Staphylococcus epidermidis Staphylococcus aureus 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[12] was used to evaluate the antimicrobial activity. Bacteria were cultured overnight at 47 ^{o} C in Mueller Hinton 10 µl Broth (MHB, Oxoid) and fungi at 58 ^{o} C for 75h 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 (4 mm in diameter) was impregnated with 10 µl of 74 µl/ml, 40 µl/ml, 54 µl/ml, 10 µl/ml and 4 µl/ml of each extracts and for each organism placed on seeded agar. Ciprofloxacin and Fluconazole (74 µl/ml, 40 µl/ml, 54 µl/ml, 10 µl/ml and 4 µl/ml) were used as positive control bacteria and fungi respectively. The test plates were incubated at 47 ^{o} C for 54h for bacteria and at 58 ^{o} C for 75h for fungi depending on the incubation time required for a visible growth (**Table 1-4**).

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[13] which was studied according to Muzushima and Kabayashi with slight modification at the doses of 500 mg/kg. The standard drug and test compounds were dissolved in minimum quantity of DMF and diluted with phosphate buffer (0.5 M, pH 7.4). Final concentration of DMF in all solutions was less than 5.4%. Test solution (1 ml) containing different concentrations of drug was mixed with 1ml of 1mM albumin solution in phosphate buffer and incubated at 57 ºc + 1 ºc in water bath for 10 min. After cooling, the turbidity was measured at 440 nm (UV – Visible Spectrophotometer SL – 149, Elico India Ltd.). Percentage of inhibition of denaturation was calculated from control where no drug was added[22]. Each experiment was done in triplicate and average is taken (Table5 and 6)

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[23]. The reaction mixture contained 40 µL of test samples (or 80% MeOH as blank) and 4 mL of a 0.004% (w/v) solution of DPPH in methanol. Different known antioxidants, vitamin E, and butylated hydroxy toluene (BHT, Sigma) were used for comparison or as a positive control. Discoloration was measured at 417 nm after incubation for 40 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 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 417 results of DPPH scavenging activity were recorded (Table 7).

RESULTS AND DISCUSSION

Table 1: Antibacterial activity of methanolic extract from extract from leaves of *Solanum xanthocarpum*

Bacterial	Extract ethanolic	Extract CCl4	Cefotax	Penicil	Tetrax
Gram (+ve)	In mm	In mm	In mm	In mm	In mm
Staphylococcus epidermidis	04	04	09	10	9
Staphylococcus aureus	04	04	10	8	8
Bacillus paludis	04	05	11	10	8
Bacillus subtilis	04	05	11	10	7
Gram (-ve)					
Escherichia Coli	04	04	7	4.4	4.4
Pseudomonas aeruginosa	04	04	4	7	4.4
Shigella flaxinely	04	04	4.4	7.4	8
Enterobacter aerogenes	04	04	4	4	5.

Table-2: Antifungal activity of Ethanolic extract from leaves of *Solanum xanthocarpum*

Fungus	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
Candida albicans	4	4	7	4	7
Aspergillus fumigatus	5	4	4	9	4
Aspergillus niger	4	04	15	8	9

Table 3: Antibacterial activity of Methanolic extract from root of *Solanum xanthocarpum*

Bacterial	Extract ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
Gram (+ve)	In mm	In mm	In mm	In mm	In mm
Staphylococcus epidermidis	14	8	7	10	9
Staphylococcus aureus	11	15	10	8	8
Bacillus paludis	15	8	11	10	8
Bacillus subtilis	14	8	11	10	7
Gram (-ve)					
Escherichia Coli	8	8	7	4.4	4.4
Pseudomonas aeruginosa	8	9	4	7	4.4
Shigella flaxinely	8	8	4.4	7.4	8
Enterobacter aerogenes	9	4	4	4	5.

Table 4: Antifungal activity of Ethanolic extract from leaves of *Solanum xanthocarpum*

Fungus	Extract Ethanolic	Extract CCl_4	Cefotax	Penicil	Tetrax
Candida albicans	4	7	7	4	7
Aspergillus fumigatus	4	9	4	9	4
Aspergillus niger	4	14	15	8	9

Table 5: 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	4ml / kg	0.098	----
Standard (Ibuprofen)	100mg/kg	0.185	84.71
Petroleum ether extract	500mg/kg	0.141	44.08
Chloroform extract	500mg/kg	0.141	44.87
Ethyl acetate extract	500mg/kg	0.154	54.44
n-Butanol	500mg/kg	0.147	70.40
Ethanol	500mg/kg	0.174	75.40

Table 6: Anti inflammatory activity of *Solanum xanthocarpum* (ethanol extract)

In-vitro Anti – inflammatory activity of cassia fistula	Dose (mg / kg)	Absorbance value (Mean + SE)	Inhibition of denaturation (%)
Control	4ml / kg	0.098	----
Standard (Ibuprofen)	100mg/kg	0.188	
Petroleum ether extract	500mg/kg	0.144	55.87
Chloroform extract	500mg/kg	0.147	55.77
Ethyl acetate extract	500mg/kg	0.151	44.44
n-Butanol	500mg/kg	0.177	4,51
Ethanol	500mg/kg	0.184	1.44

Table 7: Antioxidant activity of leaves of *Solanum xanthocarpum*

Extract Conc. Mg/ml	BHT	Ethanol	$CHCl_4$	CCl_4
0.04	44.1	15.11	09.44	08.47
0.1	44.91	11.44	15.44	09
0.5	49.54	10.54	15.40	11
0.4	47.47	20	10.00	15

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. Ethanolic extract show highest activity. Anti-inflammatory activity of *solanum xanthocarpum* has found 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 [4-7]. 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 4 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 ***Solanum xanthocarpum*** 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.

CONCLUSION

Antibacterial, antioxidant and anti-inflammatory studies of leaves and root extract of *solanum xanthocarpum* was carried out. All the extracts were tested against certain Gram positive and Gram negative organisms by well diffusion method. Anti-inflammatory activity of ethanolic and chloroform extract are maximum as well as antioxidant activity is maximum for ethanolic extract studied by DPPH Method. This study will provide a good source for future researchers to develop some new bioactive extracts.

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REFERENCES

1. Christopher D. Brickell (2008). RHS A-Z encyclopedia of garden plants. United Kingdom: Dorling Kindersley. 2008; 1136.
2. Nico Vermeulen. The Complete Encyclopedia of Container Plants: Detailed Descriptions of Hundreds of Species. Rebo Publishers. 2004; 216.
3. Kapil A, Koul IB and Suri OP. Antihepatotoxic effects of chlorogenic acid from Anthocephalus cadamb Phytother Res. 1994; 9(4): 189-194.
4. Sianne S and Fanie RVH. Antimicrobial activity of plant metabolite. Nat Prod Rep. 2005; 19: 474-495.
5. Mondal S, Dash GK and Acharyya S. Analgesic, anti-inflammatory and antipyretic studies of Neolamarckia cadamba barks. Journal of Pharmacy Research. 2009;5(4):1144-1144.
6. Mondal S, Dash GK, Acharyya A, Acharyya S and Sharma HP. Studies on diuretic and laxative activity of bark extracts of Neolamarckia cadamba (Roxb.) Bosser Drug Invention Today. 2009;1(1):78-80.
7. Sahua NP, Koike K, Zhonghua J, Banerjee S, Mondal NB and Nikaido T. Triterpene glycosides from the bark of Anthocephalus cadamba. J Chem Res. 2000;1(1):55-54.
8. Naithani HB and Sahni K C. Forest Flora of Goa, Edn. 1. International Books distributors, Deharadun, India. P. 418.
9. Kiritikar KR and Basu BD. Indian Medicinal Plants, Vol-II. Lalit Mohan Basu, Allahabad, India. 1944:1541-1545.
10. Majumdar A. Home Remedies in Ayurveda, Edn. 1. Amar granth publication, Delhi, 2005:594-597.
11. Dash GK, Mishra S, Panda A, Patro CP and Ganapathy S. Anthelmintic activity of Evolvulus nummularius. Indian Journal of Natural Products. 2004;19(4):54-54.
12. Dash GK, Suresh P, Sahu SK, Kar DM, Ganapathy S and Panda SB. Evaluation of Evolvulus alsinoides Linn for anthelmintic and antimicrobial activities. Indian Journal of Natural Remedies. 2005;5(5):185-184.
13. Ghosh T, Maity TK, Bos A and Dash GK. Anthelmintic activity of Bacopa monierri. Indian Journal 5004;51(5):14-19.
14. Bhandary MJ, Chandrashekar KR and Kaveriappa KM. Medical ethnobotany of the siddis of Uttara Kannada district Karnataka. J Ethnopharmacol. 1994; 47(4):149-148.

15. Pal DC and Jain SK. Tribal Medicine, Naya Prakash, New Delhi. 2000:45.
16. Slkar IV, Kakkar KK and Chakre OJ. Glossary of Indian Medicinal Plants with Active principles, Part 1. CSIR, New Delhi. 1995:74.
17. Brown RT and Chapple CL. Anthocephalus alkaloids: cadamine and isocadamine. Tetrahedron Letters.1974;19:459-1440.
18. Rastogi RP and Mehrotra BN.Compendium of Indian medicinal plants, Vol. II. Central Drug Research Institute, Lucknow, Publications and information directorate, New Delhi India. 1994:44-47.
19. Kokate CK. Practical Pharmacognosy, Edn. 14, Vallabh Prakashan, Delhi. 1994:107.
20. Mukherjee PK. Quality Control Herbal Drugs, Edn.1, Business Horizons Pharmaceutical Publishers, New Delhi. 5005:544-479.
21. Vidyarthi RD. Text Book of Zoolozy, 14th Edn., Chand and Company, New Delhi. 1977:459-441.
22. Thorn GW, Adams RD, Brauwald E, Isselbacher KJ and Petersdoft RG. Harrison`s Principles of Internal Medicine, Megraw Hill and Company, New York. 1977:1088-1094.
23. Vigar Z. Atlas of Medical Parasitology, 5nd Edn. P.G. Publishing House, Singapur. 1984:514.

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