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Anti-Inflammatory and Antimicrobial activity of *Flacourtia Ramontchi* Leaves

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Abstract

The literature survey revealed that a very merge amount of pharmacological work has been carried out on *Flacourtia ramontchi*. Also it was observed from the Ayurvedic literature and Ethnobotanical studies that the plant is very useful in treating inflammation and infectious diseases but no scientific investigation has been done in such direction. Very merge work has been done regarding phytochemical and pharmacological effectiveness on this plant. Successive extraction of the leaves with solvents of increasing polarity; preliminary phytochemical studies of different extracts; screening of chloroform, methanol and hydromethanolic extracts for anti-inflammatory (by Carrageenan induced rat paw model) and antimicrobial activity (by Cup and plate method) and thin layer chromatographic studies of active extracts using mobile phase i.e. chloroform and methanol. The results clearly indicate that all three extracts i.e. chloroform, methanol and hydromethanolic, of the leaves having anti-inflammatory activity. But the chloroform and methano extract showed promising results and even chloroform extract at the dose 150mg/kg exhibits equipotent anti-inflammatory activity as that of the standard Indomethacin. Methanol extract possess broad-spectrum antimicrobial activity at concentration 10000 µg/ml whereas hydromethanolic and chloroform extracts having more or less antimicrobial activity.

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Inflammation and rheumatism remain serious health problems in the present era. Current allopathic drugs are showing side effects on prolong

use. The importance of anti-inflammatory agents cannot be exaggerated because of their utility often as life saving drugs, in many diseases such as arthritis and rheumatic fever. The search for efficient anti-rheumatic drugs has been hampered by the lack of satisfactory animal models of the disease. Rheumatoid diseases are characterized by chronic inflammation, it is common practice to test compounds for anti-inflammatory activity then hopefully predict that active ones should be useful anti-rheumatic drugs. Clinically anti-inflammatory drugs are judged by their effect on the pain, stiffness or swelling of the affected part, the action on swelling being the most objectively observable, therefore most important[1,2]. Among the in- vivo methods for the evaluation of anti-inflammatory drugs, the carrageenan induced rat paw oedema assay is believed to be one of the most reliable and widely used method.

The phytochemical investigation and knowledge of the biological activities and or chemical constituents of plant is desirable not only for the discovery of new therapeutic agents but because such information may be of value in disclosing new sources of such economic material for the synthesis of complex chemical substance. Also a novel chemical structure isolated from the plant source often prompts the chemist to a successful series of modified semi synthetic compounds, which may have some or more potent medicinal and economical value. Sometimes derivatives made from isolated compounds have more potent activity than parent molecule[5,6] .There are some reasons, which suggested discovery of newer antibiotics in order to get better antimicrobial agents to treat microbial diseases and infection in future. Clinical microbiologists have two reasons to interest in the topic of antimicrobial plant extracts. First it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that, on average two or three antibiotics derived from microorganisms are launched each year[7-9]. New sources, especially plant sources, are also being investigated. Second, the public is becoming increasingly aware of problems with the over prescription and misuse of traditional antibiotics. In addition many people are interested in having more autonomy over their medical care.

Material and Methods

Test microorganism and culture media

In the present study strains used were , Gram positive bacteria *Staphylococcus aureus*, *Bacillus subtilis*, Gram negative bacteria *Escherichia coli, Proteus vulgaris*, Fungi *Candida albicans*. The bacterial cultures were grown on nutrient agar slopes for 24 h at 37°C in incubator. They were stored at 4°C and sub culturing was done after every one week. For experimental work the 18 h growth of these bacteria in nutrient broth were used. The fungal cultures were grown on sabourand dextrose agar slopes for seven days at 25°C, (room temperature) [10-12].They were stored at 4°C and sub culturing was done after one week. 72 h growth of these microorganisms in Soyabean Casein digest medium was used.

For the evaluation of antimicrobial activity following culture media were used. Nutrient Agar Medium Here 28.0 g of the dehydrate of nutrient agar medium was dissolved in one litre of distilled water and is boiled for complete dissolution. The pH of the medium was adjusted to 7.4 by adding required amount of NaOH solution. Sabourand Dextrose Agar Medium. This medium is used for the growth of fungi. 65.0 gm of the sabourand agar medium was suspended in 1 L of distilled water and boiled to obtain a clear solution. The pH of the medium was adjusted to 5.6 using required amount of hydrochloric acid solution[13,14].

Animals used

Albino rats of either sex weighing between 120-150g were used in a group of five animals. All animals were marked in position with the ink so as to dip in the cell to the same height. The test preparation was administered orally at a dose of 100, 150 and 200mg/kg body weight. The control group was given only suspension of gum acacia. After an hour 0.1 ml of 1 % carrageenan was injected into the plantar tissue of right hind paw and immediately paw volume was measured, by dipping the paw upto the mark in the cell. The paw volume was again recorded after one and three hours[16,17]. The same experiment was done using indomethacin at dose 10 mg /kg body weight. The observations are shown in Table No. 1.

The activity of the drug is expressed as percent inhibition of oedema %inhibition = 100 x (1- Vt/Vc)

Where Vt and Vc are the average increase in paw volume of drug treated and control group respectively.

Plant materials

Flacourtia ramontchi (L Herit) **(**Family Flacourtiaceae**),** The plant of *Flacourtia Ramontchi* was collected from the local region of Nagpur district (Bull Depo, Campus). The above material was botanically identified and confirmed from the Department of Botany, Nagpur University, Nagpur. The plant specimen was dried and its herbarium sheet was prepared and it is available in Department of Botany, Nagpur University, Nagpur. Specimen voucher No. is 5561/1. The leaves are useful in pruritis and scabies. The fruits are sweet appetizer, digestive and diuretic and are useful in strangury, jaundice, gastropathy and splenomegaly. The ground seeds are applied with Haldi (Turmeric) and Sonth (Dried Ginger) on woman body in the form of paste in order to reduce the body pain after delivery.The bark is applied to the body along with that Albizzia at intervals of a day or so during intermittent fever in Chota Nagpur (Campbell).

Phytochemical screening

The preliminary phytochemical screening of the residues obtained was done in view to know the various classes of chemical constituents i.e. primary and secondary metabolites. The chloroform extract showed the presence of sterols, methanolic extract showed the presence of lignans, triterpenoids, saponins and sugar whereas hydromethanolic extract showed the presence of lignans and sugar.

Preprations of extracts

The leaves of *Flacourtia ramontchi* were air dried in shade, under normal environmental conditions and then subjected to size reduction to get coarse powder. Such powdered material was charged into the Soxhlet apparatus, and extraction was carried successively with the solvents Chloroform, Methanol, Hydromethanolic. In case of extraction with hydroalcohol, maceration for 7 days was carried in cold conditions [18-20]. Each time before Extracting With the next solvent, the powdered material was air dried below 50°C and then each extract was concentrated by distilling off the solvent to obtain the crude extract (residue). The drug was extracted with each solvent till complete extraction was effected (about 30 cycles).

Preparation of solutions of the extracts

For antimicrobial activity - Before testing of these extracts for antimicrobial activity, they were completely dried at normal conditions. Dissolving them in DMSO and diluting it with sterilized water prepared the stock solutions of each extract of different concentrations .Positive control Chloramphenicol 30 µg/ml, Nystatin 30 µg/ml.

For anti-inflammatory activity - The suspension of all the three extracts and indomethacin, as standard were prepared using saline and gum acacia as suspending agent.

Culture and growth conditions

For the evaluation of antimicrobial activity culture media used were Nutrient Agar Medium .This medium is used for the growth of bacteria. 28.0 g of the dehydrate of nutrient agar medium was dissolved in one litre of distilled water and is boiled for complete dissolution. The pH of the medium was adjusted to 7.4 by adding required amount of NaOH solution. Sabourand Dextrose Agar Medium. This medium is used for the growth of fungi. 65.0 gm of the sabourand agar medium was suspended in one litre of distilled water and boiled to obtain a clear solution. The pH of the medium was adjusted to 5.6 using required amount of hydrochloric acid solution[21].

Antimicrobial activity

Antimicrobial activity was done by cup plate method. After pouring the extracts in the cups, the plates were kept in refrigerator for two hours, as a period of pre incubation diffusion. Then the plates were kept in incubator for 18 hours at a temperature of 37°C for bacteria and 7 days at room temperature in case of fungi. After incubation the diameter of the resultant growth inhibition zones were measured [22]. Each extract and antibiotic was tested against each organism in triplicates and the mean diameter of inhibition zone was tabulated.

Anti-inflammatory activity

Both in-vivo and in-vitro methods are available for the evaluation of anti-inflammatory agents but among the in-vivo methods the carrageenan induced rat paw oedema assay is believed to be one of the most reliable and also the most widely used. The oedema, which develops in rat paw after carrageenan injection, is a biphasic event. The initial phase is attributed to the release of histamine and serotonin, the oedema maintained between the 1st and 2nd phase to kinin like substances and the 2nd phase to prostaglandins like compound [23].

Paw volume measurement

The instrument used for rat paw volume measurement was digital Plethysmometer (UGO BASTILE). The instrument consists of two transparent interconnected cells. One of the cells within it is connected by two electrodes, which are connected to the reading device. The cell is marked at the upper end, which is used for dipping the rat paw. The reading device gives electronic display by which the volume of rat paw can read. A paddle switch is provided with the instrument to avoid fluctuations due to personal error of dipping the rat's paw to get the constant reading. A reservoir containing the displacement fluid is connected to the interconnected cell and kept at slightly higher level. The displacement fluid is solution of sodium chloride (.04-.05 % w / v) containing 4 to 5 ml of wetting agent. The addition of wetting agent helps to minimize the formation of drops and meniscus buildup. The concentration of sodium chloride used helps to attain the conductivity required for optimum performance. The instrument is switched on for 10 minutes to warm up before taking the reading. When the rat paw is dipped in the cell, it displaces a certain volume of solution and this volume (paw volume) is directly displayed on the reading device. The drug is administered orally to the rats and then after one hour a definite volume of carrageenan is injected into the plantar tissue of the right hind paw and immediately the paw volume is recorded. The paw volume is again recorded after one and three hours. The decrease in the paw volume as compared to the paw volume of rat receiving carrageenan only indicates the anti-inflammatory activity .

Results

In order to evaluate possible anti-inflammatory activity of *Flacourtia ramontchi* extracts, Carrageenan induced rat paw oedema test is carried out. The results clearly indicate that all three extracts i.e. chloroform, methanolic and hydromethanolic, of the leaves having anti-

inflammatory activity (Table 1). But the chloroform and methanolic extract showed promising results and even chloroform extract at the dose 150mg/kg exhibits equipotent anti-inflammatory activity as that of the standard Indomethacin (Graph 1,2,3).

By using Cup-plate technique (Diffusion Agar Method) antimicrobial activity of all three extracts i.e. chloroform, methanolic and hydromethanolic was determined and results tabulated accordingly. The results obtained shows that methanolic extract possess broad-spectrum antimicrobial activity at concentration 10000 µg/ml whereas hydromethanolic and chloroform extracts having more or less antimicrobial activity. The degree of growth of inhibition of methanolic extract of *Flacourtia ramontchi* leaves ranges from 11mm to 9mm. The inhibitory effect of extract is very close and identical in magnitude for gram positive, gram-negative bacteria and fungi (Table 2).

The extracts showing anti-inflammatory and antimicrobial activity were subjected to TLC studies to estimate number of components present in it. Thus from above results it can be said that, the leaves of *Flacourtia ramontchi* possess more promising anti-inflammatory activity and feeble antimicrobial activity.

Conclusion and future scope

In the present work, the attempt was made to study anti-inflammatory and antimicrobial activity of higher plants. For this the leaves of *Flacourtia ramontchi* were selected. And from the results it is clearly proved that leaves of *Flacourtia ramontchi* has promising anti-inflammatory activity and feeble antimicrobial activity. Scientists from divergent fields are investigating plants anew with an eye to their usefulness. A sense of urgency accompanies the search as the pace of species extinction continues. Laboratories of the world have found literally thousands of phytochemicals. More of these compounds should be subjected to animal and human studies to determine their effectiveness in whole- organism systems, including in particular toxicity studies as well as an examination of their effects on beneficial normal microbiota. In future, by isolating the various classes of secondary metabolites by different separation technique such as preparative TLC, column chromatography from these extracts and evaluating the anti-inflammatory and antimicrobial activity of the major phytoconstituents obtained from each extracts.The literature survey and preliminary phytochemical screening revealed that triterpenoids and lignans are the secondary metabolites present in active extracts of *Flacourtia ramontchi,* which may be responsible for such activity. So in future isolation of such active principles in pure form and its development can lead to a new potent anti-inflammatory and antimicrobial compound.

References

1) Bingol, F., Sener, B., "Review: Anti-inflammatory activity in plants" Herbclip, 1998.
2) Edardo, H., Arrigoni-Martelli, "Inflammatory and anti-inflammatory agents," Spectrum Publications, New York, 1977, pp887.
3) Foye, W., Lemke, T., Williams, D., "Principles of medicinal chemistry", B. I. Waverly Pvt. Ltd., New Delhi, 4th ed. 1995, pp 537.
4) Margaria, H., Rosa, M., "Evaluation of the anti-inflammatory and analgesic activity of *Sideritis canariensis* Var. pannosa in mice," *J. Ethnopharmacology* 2002 (81): 43-47.
5) Chopra, R., Nayar, S., Chopra, I. "Glossary of Indian Medicinal Plants", (Council of Scientific and Industrial Research, New Delhi), 1992, 3rd ed. pp 94.
6) Clark, A., "Natural products as a source for new drugs," *Pharm. Res.* 1996 (13): 265-72.
7) Alper, J., "Effort to combat microbial resistance lags," ASM News 64:440-1.
8) Chattopadhyay, D., Arunachalam, G., Mandal, A., "Antimicrobial and anti-inflammatory activity of folkre *Mallotus peltatus* leaf extract," *J. Ethnopharmacology* 2002 (82): 229-37.

9) Chopra, R., Chopra, I., Handa, K., Kapur, L., "Indigenous drugs of India," Calcutta; Dhar U. N. and sons Pvt. Ltd.1958.
10) Bhaumik, P., Guha, K., Biswas, G., Mukherjee, B., " Flacourtin, a phenolic glucoside ester from *Flacourtia indica*", *Phytochemistry*, Jan 1987.
11) Cowan, M., "Clinical Microbiology Review", 1999 (12): 564-82.
12) Khandelwal, K.,"Practical Pharmacognosy", Nirali Publication, 2nd ed. August 2000, pp. 149.
13) Hufford, C., Funderburk, M., Morgan, J., "Two antimicrobial alkaloids from heartwood of *Liriodendron tulipifera*, *J. Pharma.Sci.* 1975 (5): 789.
14) Kulkarni, S., "Handbook of experimental pharmacology", Vallabh Prakashan, New Delhi, 2nd ed. 1993 pp82.
15) Orient Longman, "Indian Medicinal Plants", Vol.II pp.43
16) Moreman, D., An analysis of the food plants and drug plants of native North America, *J. Ethnopharmacology* 1996 (52): 1-22.
17) Seingle, K., "Medicinal chemistry: Anti-inflammatory Agents," Vol.XIII, Scherrer, R. and Whitehouse, M., Academic Press, New York 1974 pp 32.
18) Berry, J., Berr, P., Rodriguez E., "Benctop bioassays: Methods in chemical prospecting," L.H. Bailey Hortorium, Cornell Univers.Ithaca, NY.
19) Khandelwal, K., "Practical Pharmacognosy", Nirali Publication, 2nd ed. Aug 2002, pp 19-21.
20) Michael, G., Medical Pharmacology, Pharmacology content, 1997-2003.
21) Navarro, A., Heras, B., Villar, A. "Anti-inflammatory and immunomodulatory activity of sterol fraction from *Sideritis foetens*," *Biol. Pharm. Bull.*2001 (5); 470-473.
22) Pankaj Oudhia, Research Note, "Medicinal herbs of Chhattisgarh, India having less known traditional uses."
23) Pelzar, M., Chan, E. and King, N., " Microbiology" Mcgraw hills 5th ed., 1986.