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Anti-Inflammatory Activities of Some Medicinal Plants With Special Reference *Euphorbia tithymaloides*(L.).*Luffacylindrica* (M. Roem.).*Cocciniagrandis*(L.) *Voigt*

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Abstract - Various nonsteroidal anti-inflammatory drugs have been shown to reduce pain and inflammation by blocking the metabolism of arachidonic acid by isoform of cyclooxygenase enzyme, thereby reducing the production of prostaglandin. Sadly, there are many side effects associated with the administration of nonsteroidal anti-inflammatory drugs. However, there are medicinal plants with anti-inflammatory therapeutic effects with low or no side effects. The Afri-can continent is richly endowed with diverse medicinal plants with anti-inflammatory activities that have been shown to be effective in the treatment of inflammatory conditions in traditional medicine. Interestingly, scientists have examined some of these African medicinal plants and documented their biological and therapeutic activities. Unfortunately, medicinal plants from different countries in Africa with anti-inflammatory properties have not been documented in a single review paper. It is important to document the ethnobotanical knowledge and applications of anti-inflammatory medicinal plants from selected countries representing different regions of the African continent. This paper therefore documents anti-inflammatory activities of various medicinal plants from different geographical regions of Africa with the aim of presenting the diversity of medicinal plants that are of traditional or therapeutic use in Africa.

Keywords: inflammation, inflammatory conditions, local plants, plant potentials.

Introduction - Reports have shown that inflammation is usually triggered by damage to living tissues resulting from bacterial, viral, fungal infections; physical agents; and defective immune response. The fundamental aim of inflammatory response is to localize and eliminate the harmful agents; secondarily, to remove damaged tissue components to culminate in healing of the affected tissues, organs, or system.An inflammatory response involves macrophages, neutrophils known to secrete different mediators that are responsible for the initiation, progression, persistence, regulation, and eventual resolution of the acute state of inflammation. The resolution of inflammation is influenced by several anti-inflammatory mediators and the recruitment of monocytes for the removal of cell or tissue debris. It is possible that the resolution may not occur in the acute phase, thereby turning into a chronic phase. Chronic inflammation plays a role in the burdens associated with pathological conditions in both developed and developing countries, particularly in African countries. For instance, chronic inflammation is known to play a role in the development of obesity-associated diabetes secondary to insulin resistance Various nonsteroidal anti-inflammatory drugs can reduce pain and inflammation by blocking the metabolism of arachidonic acid by isoform of cyclooxygenase enzyme (COX-1 and/or COX-2), thereby reducing the production of prostaglandin. Unfortunately, there are many side effects associated with the administration of nonsteroidal anti-inflammatory drugs. However, there are medicinal plants with anti-inflammatory therapeutic effects with low or no side effects. The African continent is richly endowed with diverse medicinal plants with anti-inflammatory activities that have been shown to be effective in the treatment of inflammatory conditions in traditional medicine. Interestingly, scientists have examined some of these African medicinal plants and documented their biological and therapeutic activities. Unfortunately, medicinal plants from different regions in Africa with anti-inflammatory properties have not been documented in a single review paper. Therefore, it is important to document the ethnobotanical knowledge and applications of anti-inflammatory medicinal plants from selected countries representing different regions in the African continent. For centuries, Africans have treated different disease conditions including inflammatory diseases using medicinal plants. Africa is a vast continent. From Egypt, Morocco, and Algeria in the north to Nigeria and Ghana in the west, Cameroon

and Gabon in the center, Kenya and Tanzania in the east, and South Africa, Lesotho, Namibia, Swaziland, and Zimbabwe in the south, there are thousands of plants with therapeutic values. For example, there are over 5000 plant species growing in Zimbabwe with over 10% of these plants having medicinal value while in South Africa over 30000 flowering plants are available and some of them are used in treatment and management of pain-related inflammatory disorders in both animal and human subjects. The truth is that African traditional medicine is usually the first contact in meeting the primary health care need in Africa and is related to its affordability, accessibility, cultural and spiritual acceptance, and knowledge of its preparations and use.The potentials of plant-derived compounds from African medicinal plants have been reported and the interest to use medicinal plants in treatment and management of disease conditions is growing rapidly in Africa even among educated African urban dwellers.Medicinal plants with anti-inflammatory activities from countries and regions of Africa

Materials and methods

Materials : The COX-1 & 2 (human ovine) inhibitor Screening assay kit [Catalog No. 760111] was procured from Cayman, U.S.A., MTT (3- (4, 5-dimethylthiazol-2-yl) – 2, 5- diphenyl tetrazolium bromide), DPPH (1, 1-diphenyl-2-picryl hydrazyl) were purchased from Sigma-Aldrich Co. (St. Louis MO, USA). 1–10 phenanthroline, Phenazine methosulphate (PMS), Nitroblue tetrazolium (NBT) were obtained from s.d. Fine chem. Mumbai. Nicotinamide Adenine Dinucleotide (NADH) was purchased from Spectrochem, Pvt. Ltd. Mumbai. Chang Liver cell line was requested from National Centre for Cell Science (NCCS: a National Cell Line Facility) Pune (MS), India Medicinal plants were collected from the nearby areas of Nanded district (MS), India. All other chemicals and reagents used were of AR grade and were obtained from commercial sources. Collection and identification of the selected medicinal plants ***Euphorbia tithymaloides*(L.). *Luffacylindrica*(M. Roem.).*Cocciniagrandis*(L.) *Voigt*** were collected from the nearby regions of district . The plants were identified and authenticated with the help of Flora. and Voucher specimens (A13-A17) of the plants were deposited in the herbarium center of Department of Botany, School of Life Sciences, bu bhopal. The shade dried and powdered plant samples were preserved for further experimentations.

Sequential extraction of the plant samples: The shed dried powdered plant samples (10 g) were sequentially extracted in hexane, ethanol and water up to 8 h using Soxhlet's apparatus. The extracted samples were evaporated under reduced pressure at room temperature. The dried extracts were preserved at 4 °C in refrigerator for further analysis.

HPTLC analysis: HPTLC analysis was performed using CAMAG (Germany) make instrumental thin layer chromatography. TLC plates (Merck silica gel 60 F254, 20 cm × 10 cm) were prewashed with methanol. The plate was activated in an oven at 100 °C for 10 min. Individual plant extracts of 10 µl (1 mg/ml) were spotted onto the precoated plates using a Linomat 5 application system. Rutin hydrate (5 and 10 µg/ml) was used as a marker flavonoid. The flavonoids were separated using ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:27) as a mobile phase. Natural product (NP) reagent was used as a flavonoid derivatizing agent and the spots developed were visualized under CAMAG UV cabinet (366 nm) and were digitized using CAMAG photo documentation system.

COX inhibition assay: The assay was performed by using Colorimetric COX (human ovine) inhibitor Screening assay kit. Briefly, the reaction mixture contains, 150 µl of assay buffer, 10 µl of heme, 10 µl of enzyme (either COX-1 or COX-2), and 10 µl of plant sample (1 mg/ml). The assay utilizes the peroxidase component of the COX catalytic domain. The peroxidase activity was assayed colorimetrically by monitoring the appearance of oxidized N, N, N, N'-tetramethyl-*p*-phenylenediamine (TMPD) at 590 nm. Aspirin (acetylsalicylic acid, 1 mM) was used as a standard drug. The percent COX inhibition was calculated using following equation:

COXinhibitionactivity(%)=1-TC×100

Where,

T = Absorbance of the inhibitor well at 590 nm.

C = Absorbance of the 100 % initial activity without inhibitor well at 590 nm.

DPPH radical scavenging assay: DPPH radical scavenging assay was carried out as per reported method with slight modifications. Briefly, 1 ml of test sample (1 mg/ml) was added to equal quantity of 0.1 mM solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1 mM) was used as reference compound.

Hydroxyl (OH) radical scavenging assay: The OH radicals scavenging activity was demonstrated with Fenton reaction. The reaction mixture contained, 60 µl of $FeCl_2$ (1 mM), 90 µl of 1–10 phenanthroline (1 mM), 2.4 ml of phosphate buffer (0.2 M, pH 7.8), 150 ìl of H_2O_2 (0.17 M) and 1.5 ml of individual plant extract (1 mg/ml). The reaction was started by adding H_2O_2. After 5 min incubation at room temperature, the absorbance was recorded at 560 nm. Ascorbic acid (1 mM) was used as reference compound.

Superoxide radical (SOR) scavenging assay: The SOR scavenging assay was performed by the reported method. Superoxide anion radicals were generated in a non-enzymatic Phenazine methosulphate – Nicotinamide Adenine Dinucleotide (PMS – NADH) system through the reaction of PMS, NADH and Oxygen. It was assayed by the reduction of Nitroblue tetrazolium (NBT). In this experiment superoxide anion was generated in 3 ml of Tris HCL buffer (100 mM, pH 7.4) containing 0.75 ml of

NBT (300 µM), 0.75 ml of NADH (936 µM), and 0.3 ml of plant sample (1 mg/ml). The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature the absorbance at 560 nm was measured in spectrophotometer. Ascorbic acid (1 mM) was used as reference compound.

MTT cytotoxicity assay: The MTT cytotoxicity assay was performed as published previously.42, 43, 44 The Chang liver cells were harvested (1.5×10^4 cells/well) and inoculated in 96 well microtiter plates. The cells were washed with phosphate buffered saline (PBS) and the cultured cells were then inoculated with and without the individual ethanolic plant extract (1 mg/ml). After 72 h incubation, the medium was aspirated followed by addition of 10 µL of MTT solution (5 mg/ml in PBS, pH 7.2) to each well and the plates were reincubated for 4 h at 37 °C. After incubation time, 100 ìL of DMSO was added to the wells followed by gentle shaking to solubilize the formazan crystal for 15 min. Absorbance was read at 540 nm using Thermo make Automatic Ex-Microplate Reader (M51118170) and the % cell viability was calculated. The H_2O_2 (1 mM) was used as reference cytotoxic agent. The percent DPPH, OH, SOR scavenging activity and cell viability inhibition was calculated using following formula.

Inhibitionactivity(%)=1-TC×100,Where T = Absorbance of the test sample.

C = Absorbance of the control sample.

Experimental animals

The animals used in this study were Swiss albino mice weighing between 25–30 g. They were maintained in experimental animal house at Sudhakarrao Naik Pharmacy College, Pusad (MS), India. They were kept in rat cages and fed on standard mice food (Amrut Feeds Ltd., Sangali (MS), India) and allowed free access to clean fresh water in bottles. The experimental protocols were in compliance with Institutional Animal Ethics Committee (IAEC), Sudhakarrao Naik Pharmacy College, Pusad (MS), (Proposal No. CPCSEA/IAEC/PL/09–2011).

Carrageenan-induced rat paw edema assay: The selected samples showing promising average (activity in all solvents) COX-2 selective activities were evaluated for *in vivo* anti-inflammatory studies using carrageenan induced rat paw edema animal model. The assay performed as described previously.Briefly, edema was induced on the right hind paw by subplantar injection of 20 µl carrageenan (1% w/v) in 0.9 % saline. The extract of selected samples were prepared in 1 % w/v gum acacia and administered orally at a dose of 100 mg/kg and 250 mg/kg, 1 h before carrageenan injection. A control group received vehicle only and a standard group was treated with indomethacin (20 mg/kg, p.o.) The volume of injected and of the contra lateral paws was measured 1, 3, and 5 h after induction of inflammation, using a plethysmometer (Orchid Scientific Laboratory). The value was expressed as, the percent reduction in volume with respect to the control group of at different time intervals.

PMA induced mouse ear edema activity: According to a modified method of 4 µg per ear of PMA, in 20 µl of acetone, was applied to both surfaces of the right ear of each mouse. The left ear (control) received the vehicle (acetone and/or DMSO, 20 µl). The selected plant extract was administered topically (50 and 100 mg per ear in DMSO) 1 h before PMA application. Two control groups were used: a control group with the application of PMA on the right ear and the reference group was treated with indomethacin (2 mg per ear in 20 µl acetone). Six hours after PMA application, mice were killed by cervical dislocation and a 6 mm diameter disc from each ear was removed with a metal punch and weighed. Ear edema was calculated by subtracting the weight of the left ear (vehicle) from the right ear (treatment), and was expressed as edema weight. Inhibition percentage was expressed as a reduction in weight with respect to the control group.

Results

HPTLC profiling: The HPTLC analysis was performed as a part of quality control of the selected plant samples. HPTLC finger print of ethanol soluble flavonoids was prepared using rutin as a marker flavonoid compound The results of the HPTLC analysis shows the diversity of flavonoid content in *T. chebulla*, moreover this is the only sample containing rutin, while all other samples were devoid of rutin content.

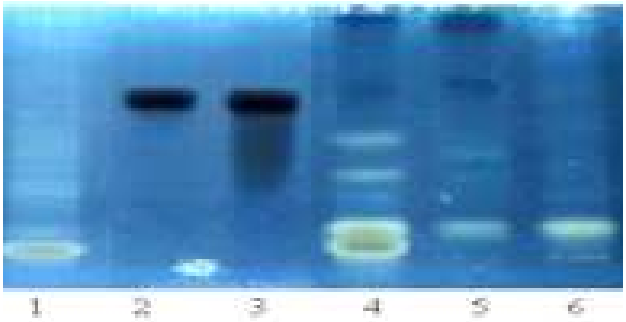

Fig. HPTLC profile of flavonoid finger prints of ethanol extract of selected medicinal plants using Rutin as a marker compound.

Euphorbia tithymaloides*(L.).*Luffacylindrica*(M. Roem.).*Cocciniagrandis*(L.) *Voigt

Effect of plant samples on COX inhibition

OH radical scavenging activity: The profile of OH radical scavenging activity of selected medicinal plants. Except water extract all samples are found to be promising OH radical scavengers. The ethanol extract possess maximum activity while ethanol extract of *e.u*(38.05 ± 0.77 %) showed poor OH radical scavenging ability as compared to ascorbic acid (2.63 ± 0.73 %)

SOR scavenging assay: The water and ethanolic extract of all selected plants showed promising SOR scavenging activity as compared to hexane extract. The high SOR scavenging activity was found in ethanolic extract of *P. zeylanica* (57.21 %) while the lower SOR was reported

in hexane extract of *C. quadrangularis* (0.79 %.). From the tested samples it was observed that the SOR scavenging activity was recorded in the range of 3.81–34.55 %. The results were compared with ascorbic acid (52.95 ± 0.83 %).

cytotoxicity assay: It was observed that none of the plant sample showed significant cytotoxic effect on normal Chang liver cell viability at 1 mg/ml concentration. The observed and calculated percent inhibition of cell viability .

Profile of carrageenan induced anti-inflammatory activity:value for respective time interval (One Way ANOVA for multiple comparison test followed by dunnet test).

Oedama induced by PMA in mouse ear activity: It can be seen in that the selected plant samples inhibit PMS indued inflammation in mouse ear. The extracts of *e.u* (54.06 %) and *c.c*(47.84 %) shows significant ($P < 0.05$) inhibitory results as compare to *P.h*(30.21 %) at the higer dose (100 mg/ear). Indomethacin was used as reference compound also possess an excellent inhibitory activity.

Discussion: Developing novel, effective and safe anti-inflammatory agents has remained a major thrust area in the main stream of 'finding alternatives to NSAID's. Anti-inflammatory agents possessing selective COX-2 inhibition and showing no or negligible effect on COX-1 activity are more appreciated as safe drugs as these agents have minimum gastrointestinal side effects. Natural product, especially medicinal plants and drug discovery has remained a very successful combination for the inventorization of new therapeutic agents. The main intention of the present study was to perform the COX activity guided standardization of selected medicinal plants with focus on antioxidant and cytotoxicity profile. Variety of phytochemicals like flavonoids, terpenoids, alkaloids and saponins has been described to possess significant anti-inflammatory activity.

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