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Research Article

Pharmacological Screening of Combination of *Alpinia officinarum and Solanum Xanthocarpum* Herbal Plants for Antiinflamatory Activity

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Abstract

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones. Herbal formulations have growing demand in the world market. *Solanum xanthocarpum* herb is highly used by the rural and tribal people in curing various disorders. The aim of the current investigation is evaluation of anti inflammatory activity of *solanum xanthocarpum* extract and *Alpinia officinarum. In-vitro* anti-inflammatory study performed by percentage inhibition of Human red blood cell (HRBC) membrane stabilization method. Four different concentration of extract 1mg/ml, 2 mg/ml, 4 mg/ml and 6 mg/ml were used for each extract. Among which ethanolic extract of *S. xanthocarpum* at concentration 6 mg/ml showed 49 % protection of HRBC in hypotonic solution and *A. officinarum* extract at concentration 6 mg/ml showed 53.89 while combination of extract (1:1 ratio) at concentration 6 mg/ml showed 65.42 % protection of HRBC in hypotonic solution. All the results were compared with standard indomethacin which showed 69.0 % protection at concentration 2.5 mg/ml

Keyword : Natural remedies, anti-inflammatory, Human red blood cell (HRBC) membrane stabilization, hypotonic solution

Introduction :

Medicinal plants contain inherent active ingredients to cure disease or relieve pain. The use of traditional medicines and medicinal plant in most developing countries as therapeutic agent for the maintenance of good health has been widely observed. The world health organization estimated that 80% of the population of developing countries relies on traditional medicines, mostly herbal plant drugs for their primary health care. The medicinal property of plant could be based on the anti-oxidant, antimicrobial, antipyretic effect of the phytochemicals present. Traditionally, herbs have been considered to be non-toxic and have been used for treating various problems by the general public and/or traditional medicine doctors worldwide. Although, the literature has documented several toxicity resulted from the use of herbs on many occasions, still the potential toxicity of herbs has not been recognized by the general public or by professional groups of traditional medicine. The use of medicinal plants as raw materials in the production of drug is gaining popularity.[1,2]

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. Alpinia officinarum (known as lesser galangal) is native to the island of Hainan, in the Southern part of China .The rhizome is the most commonly used part of this plant and has a pungent, spicy taste and an aromatic odour .Lesser galangal has a long history of traditional medicinal use in China owing to its significant therapeutic properties with respect to the spleen and stomach. It also has a reputation as a remedy for chronic gastritis. Of the many compounds identified in lesser galangal, diarylheptanoids are among the most important. Several diarylheptanoids have been isolated from this plant and found to display strong anti-oxidative activities.

Solanum xanthocarpum (SX) Schrad. & Wendl. (Family: Solanaceae) commonly known as the Indian night shade or Yellow berried night shade (English) and kantakari (Sanskrit). It is a prickly diffuse, bright green perennial herb, woody at the base, 2–3 m height, found through out India, mostly in dry places as a weed along roadsides and waste lands . SX has held a place of some importance in the Hindu *Materia Medica*, primarily as an expectorant and antipyretic. Various medicinal properties are attributed to it, particularly in the treatment of asthma, chronic cough and catarrhal fever.[3-6]

Materials and Methods[7-14]

Collection of plant: *Ipomoea carnea* The plant *Solanum xanthocarpum* and *Alpinia officinarum* were collected from Bhopal and was authenticated by Dr. S. N. Dwivedi, Prof. & Head, Department of Botany, Janata PG College, APS, University, Rewa, M.P.

Preparation of plant powder

The plant was dried under shade and then powdered coarsely with a mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for further use.

Preparation of extracts:

The dried powder of plant was extracted with various solvents. Aqueous extract was prepared by cold maceration process. Ethanolic, Methanol, chloroform and petroleum ether were obtained using Soxhlet apparatus.

About 250 gm of *S. xanthocarpum* dried fruit and *250* gm *A. officinarum* dried root powder of plant was subjected to soxhlation. It was first defatted with petroleum ether then exhaustively extracted with solvent in a Soxhlet apparatus for 36 hours. The temperature was maintained at 40-50 degree centigrade. Ethanol solvent is used for *S. xanthocarpum* extraction and methanol solvent for *A. officinarum.* The solvents were removed by distillation

under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract.[5-6 ,117]

In-vitro Anti-inflammatory activity of Plants Extracts[123-126]

In-vitro Anti-inflammatory activity of Plants Extracts[123-126] :

Ethanol extract of S. *xanthocarpum* and methanolic extract of *A. officinarum* were investigated for *In-vitro* Anti-inflammatory activity by human red blood cell membrane stabilization method. Four different concentrations of extracts: 1mg/ml, 2mg/ml, 4mg/ml and 6mg/ml were used for anti-inflammatory study.

Preparation of drug

Standard drug (Indomethacin, 2.5 mg/ml) and extracts (1.0 -6.0 mg/ml) were prepared in isosaline (0.85% NaCl) to final concentration.

Preparation of Suspension (10% v/v) of Human Red Blood cell

The blood sample was collected from healthy human volunteer who has not taken any NSAID for 2 weeks prior to the experiment and transferred to heparinized centrifuge tube. Blood samples were centrifuged at 3000 rpm at room temperature for 15 min. The supernatant (plasma and leucocytes) were carefully removed while the packed red blood cells were washed with fresh normal saline (0.85% w/v NaCl). The process of washing and centrifugation was repeated five times until the supernatant was clear. Then, Human erythrocytes suspension (10% v/v) was prepared as reported by Oyedapo et al., 2004.

Assay of Membrane stabilizing activity

The HRBC membrane stabilizing activity assay was carried out as reported by Sadique et al., 1989; Oyedapo et al., 2004 using 10% (v/v) Human erythrocyte suspension while Indomethacin was used as standard drugs. The assay mixtures consisted of 2 ml of hyposaline (0.25% w/v) sodium chloride, 1.0 ml of 0.15 M sodium phosphate buffer, pH 7.4, 0.5 ml of 10% (v/v) human erythrocyte suspension, 1.0 ml of drugs (standard and extracts) and final reaction mixtures were made up to 4.5 ml with isosaline.

To determine the anti-inflammatory activity by HRBC membrane stabilization method, the following solutions were prepared.

a) **Test solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v), 1ml of phosphate buffer (pH7.4), and 1ml of test extract (1mg/ml – 6 mg/ml) in normal saline and 0.5ml of 10% w/v human red blood cells in isotonic saline.

b) **Test control** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of isotonic saline and 0.5ml of 10%w/v human red blood cells in isotonic saline.

c) **Standard solution** (4.5ml) consists of 2ml of hypotonic saline (0.25%w/v) 1ml of phosphate buffer (7.4pH) and 1ml of Indomethacin (2.5mg/ml) and 0.5ml 10%w/v human red blood cells in isotonic saline.

Drug was omitted in the blood control, while the drug control did not contain the erythrocyte suspension. The reaction mixtures were incubated at 37°C for 30 min and centrifuged at 3000 rpm for 20 min. The absorbance of the supernatant solution was measured spectrophotometrically at 560 nm. Each experiment was carried out in triplicate and the average was taken. The percentage inhibition of haemolysis or membrane stabilization was calculated using the following equation.

$$\% \text{ Inhibition of haemolysis} = 100 \times (A_1 - A_2/A_1)$$

Where:

A_1 = Absorption of hypotonic buffered saline solution alone

A_2 = Absorption of test sample in hypotonic solution

In-Vivo Evaluation Methods Of Anti-Inflammatory Action

Inflammation is one of the most important natural defense mechanisms. Its main purpose is to destroy the injurious agent and/or to minimize its ill effects by limiting its spread. Though inflammation is protective in some situations if untreated can lead to serious complications. Inflammation is the dynamic pathological process consisting of a series of interdependent changes. Both in-vitro and in-vivo methods are commonly used or the evaluation of anti-inflammatory activity in laboratory practice.

5.4.1 *In-vivo* Anti-inflammatory study of optimized formulation[189-202]

Formalin-induced Paw Edema

This model based upon the ability of test drug to inhibit the edema produced in the hind paw of the mice after injection of formalin. The nociceptive effect of formalin is biphasic, an early neurogenic component followed by a later tissue-mediated response. In the first phase there is release of histamine, 5-HT and kinin, while the second phase is related to the release of prostaglandins

Animals

Healthy young adult albino (100-120 gm) of either sex and of approximate same age were used throughout the study were housed under standard laboratory conditions in polyacrylic cages, and were provided with pelleted food and water *ad libitum*. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. Animal studies were approved by Institutional Animal Ethics Committee (IAEC) and carried out in accordance with the Guidelines of the

Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA)

Formalin induced paw edema model

In-vivo Anti-inflammatory study of combination of extract containing *S. xanthocarpum and A. officinarum* plant extract was conducted by formalin induced paw edema model using 12 albino rats and divided into three groups of four animals on each. In all groups, acute inflammation was induced by sub-planter injection of 0.1 ml of freshly prepared 1 % suspension of sterilized formalin in normal saline in left hind paw of the rats. The medicated formulations (0.3g) or base or standard were applied topically to the planter surface of hind paw with gentle rubbing with index finger to each rat of respective group one hour before and one hour after the formalin challenge. The paw edema volume was measured using plethysmometer at every 30 mint intervals for 4 hour after injection of formalin. The average paw edema volume of all the groups were calculated and compared with that of control. The percent inhibition of edema was calculated by using following formula.

$$\% \text{ Edema inhibition} = (1\text{-}Vt/Vc)\ 100$$

Where, Vt = Mean edema volume of test, Vc = Mean edema volume of control

Eight groups of animals four each:

- Group I -Received gel base
- Group II -Received combination of extract containing *S. xanthocarpum and A. officinarum* plant extract (1:1 ratio)
- Group III- Received diclofenac (Voveran Emulgel)

Statistical Analysis

The statistical analysis of various studies were carried out using analysis of variance (ANOVA) followed by Dunnett's 't' test and standard deviation, $p<0.05$ was accounted significant.

Skin Irritation Study

In-vivo skin irritation study was conducted by 15 albino rats of either sex weighing between (100-120 g) was used. Animals were divided in to 3 groups of 5 animals on each. Hairs were depleted from the back of rats with the help of depilatories and area 2 cm^2 was marked on both the sides. One side served as control while the other as test and animals were used after 24 hrs. After hair depletion herbal extract was applied (500mg / rat) on test side and gel base was applied on control side once a day for 7 days and site was covered with cotton bandage and observed for any sensitivity and the reaction if any was graded as under [203]:-

A – No reaction, B – Slight patchy erythema, C – Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

Results and discussion

Extraction

The dried powder of plants were extracted with various solvents i.e., water, ethanolic, chloroform, petroleum ether and methanol. The solvents were removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator to obtain the extract. The percentage yields of various extract was presented in Table 1&2

Table 1: Extractive values of *Solanum xanthocarpum*

Sr. No.	Solvents	Extractive values (%w/w)
1.	Pet-ether	2.62
2.	Water	17.2
3.	Chloroform	6. 9
4 .	Ethanol	15.5
5.	Methanol	14.5

Table 2: Extractive values of *A. officinarum* rhizome.

Sr. No.	Solvents	Extractive values (%w/w)
1.	Pet-ether	0.60
2.	Water	1.6
3.	Chloroform	1.2
4.	Ethanol	1.8
5.	Methanol	2.7

Pharmacological Screening :

***In-vitro* Anti-inflammatory activity of Extracts**

.In the present study, stabilization of erythrocyte membranes exposed to both heat and hypotonic induced lyses was employed due to its simplicity and reproducibility. The ethanolic extract of the root of *S. xanthocarpum and* Methanolic extract *A. officinarum* were studied for in vitro anti-inflammatory activity by HRBC membrane stabilization method. Four different concentration of extract 1mg/ml, 2 mg/ml, 4 mg/ml and 6 mg/ml were used for each plant extract. Among which ethanolic extract of *S. xanthocarpum* at concentration 6

mg/ml showed 49.0 % protection of HRBC in hypotonic solution and *A. officinarum* extract at concentration 6 mg/ml showed 53.89% while combination of extract (1:1 ratio) at concentration 6 mg/ml showed 65.42% protection of HRBC in hypotonic solution. All the results were compared with standard indomethacin which showed 69.0 % protection at concentration 2.5 mg/ml (Table 3,.4 & Fig .1) .The activity may be due to the presence of one or more phytochemical constituents present in the extract.

***In-Vivo* Anti-Inflammatory Study of Extract**

Percentage inhibition of edema by combination of extract containing S. *xanthocarpum and A. officinarum* extracts containing 1: 1 ratio was observed to be- 25.50±0.10 (at 1 hr.) and 44.32±0.23 (at 4 hr.). All the results were compared with standard voveran emulgel gel.

The result revealed that containing both plants extract has potent anti-inflammatory action. (Table.7 & fig.2).

Skin Irritation Study

The skin irritation test was conducted for a period of seven days and the results are tabulated in Table 6.8. The results indicated that the control preparation, combination of Extract containing both plant extract and marketed products did not cause any skin reaction. It can be assured that both plants extract did not cause any skin irritation and can be used.

Table 3: *In-Vitro* anti-inflammatory activity of Ethanolic extract of *S. xanthocarpum* by membrane stabilization method

Treatment	Con(mg/ml)	Absorbance(560nm)	% of Inhibition
Control	-	0.250±0.29	-
Extract of S. xanthocarpu	1.00 2.00 4.00 6.00	0.208±0.25 [a] 0.182±0.22[a] 0.147±0.28[b] 0.123 ±0.42[c]	16.80 29.20 44.10 49.0
Indomethacin (Standard drug)	2.50	0.070±0.18[b]	69.0

Values are expressed as X (Mean) ±SEM, n=3. (One way ANOVA followed by Student t-test). Statistically significance of [a]P < 0.05, [b]P<0.01, [c]P<0.001 and [d]NS in comparison to respective control.

Table 4. *In-Vitro* anti-inflammatory activity of Methanolic extract of *A. officinarum* by membrane stabilization method

Treatment	Con(mg/ml)	Absorbance(560nm)	% of Inhibition
Control	-	0.250±0.29	-
Methanolic extract of *A. officinarum*	1.00 2.00 4.00 6.00	0.202±0.25 [a] 0.175±0.12[a] 0.143±0.23[a] 0.110±0.44[c]	18.35 30.87 46.96 53.89
Indomethacin (Standard drug)	2.50	0.070±0.18[b]	69.0

Values are expressed as X (Mean) ±SEM, n=3. (One way ANOVA followed by Student t-test). Statistically significance of [a]P < 0.05, [b]P<0.01, [c]P<0.001 and [d]NS in comparison to respective control.

Table 5: *In-Vitro* anti-inflammatory activity of combination of extract by membrane stabilization method

Treatment	Con(mg/ml)	Absorbance(560nm)	% of Inhibition
Control	-	0.250±0.29	-
Ethanolic extract of *S. xanthocarpum and* Methanolic extract of *A. officinarum* in 1:1 ratio	6.00	0.081±0.39[b]	65.42
Indomethacin (Standard drug)	2.50	0.070±0.18[b]	69.0

Table 6: Mean Percentage inhibition of edema
% Inhibition (Mean±SEM)

Group	Percentage inhibition of edema			
	1 hr	2hr	3hr	4hr
Control	-	-	-	-
Combination of extract (1:1)	25.50±0.10	29.87±0.43	37.23±0.04	44.32±0.23
Standard Drug (Voveran Emulgel)	40.02±0.16	44.00±0.03	49.10±0.05	51.85±0.90

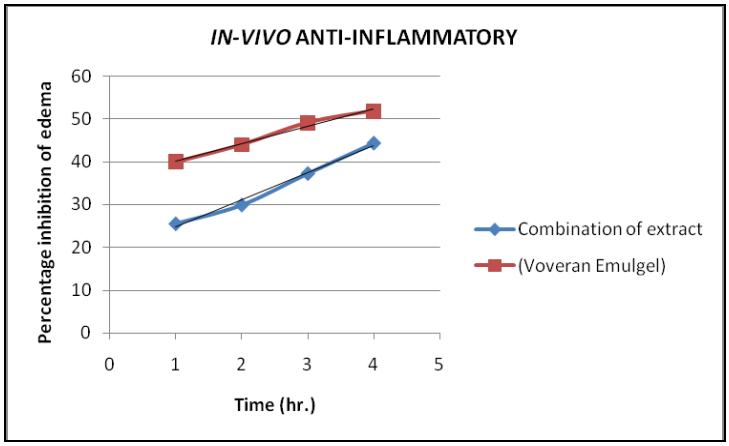

Figure 1: Percentage inhibition of edema

Table 7.: Skin irritation study

Treatment	*Day 1*	*Day 2*	*Day 3*	*Day 4*	*Day 5*	*Day 6*	*Day 7*
Control	*A*	*A*	*A*	*A*	*A*	*A*	*A*
Combination of extract (1:1)	*A*	*A*	*A*	*A*	*A*	*A*	*A*
Voveran emulgel	*A*	*A*	*A*	*A*	*A*	*A*	*A*

A – No reaction, B – Slight patchy erythema, C –Slight but confluent or moderate but patchy erythema, D – Moderate erythema, E – Severe erythema with or without edema.

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Conclusions

Percentage inhibition of edema by extract containing S. *xanthocarpum and A. officinarum* both extracts containing was observed to be- 25.50±0.10 (at 1 hr.) and 44.32±0.23 (at 4 hr.). All the results were compared with standard voveran emulgel gel. The result revealed that both plants extract has potent anti-inflammatory action and this preparation can be explored as potential anti-inflammatory product in pharmaceutical market.

Natural remedies are more acceptable in the belief that they are safer with fewer side effects than the synthetic ones All the studies performed provides a strong evidence for the use of the both plants as an anti-inflammatory agent and that can be used as an alternative remedy for management and treatment of inflammation related disorder and disease.

Reference

1. Quaye Osbourne, Cramer Precious, Ofosuhene Mark, Okine Laud K N, and Nyarko Alexander K. Acute and Subchronic Toxicity Studies of Aqueous Extract of *Desmodium adscendens* (Sw) DC. (2017); Journal of Evidence-Based Complementary & Alternative Medicine; 22(4): 753-759.
2. Saleem Uzma, Amin Sadia, Ahmad Bashir, Azeem Haroon, Anwar Fareeha, Mary Sunita. Acute oral toxicity evaluation of aqueous ethanolic extract of *Saccharum munja* Roxb. roots in albino mice as per OECD 425 TG. (2017); Toxicology reports; 1-20.
3. Upadhyay Prabhat, Shukla Rashmi, Mishra Sunil Kumar. Acute and sub-acute toxicity study of hydro-alcoholic leaves extract of *Reinwardtia indica* in rats. (2019); Biomedicine & Pharmacotherapy; 111: 36–41.
4. Al-Yahya M.A., Rafatullah S., Mossa J.S., Ageel A.M., Al-Said M.S., Tariq M., et al. (1990) "Gastric antisecretory, antiulcer and cytoprotective properties of ethanolic extract of *Alpinia galanga* Willd in rats" Phytotherapy research 4(3): 112-114.
5. An N., Zou Z., Tian V, Luo X, Yang S, Xu LET., et al. (2008) "Diarylheptanoids from the rhizomes of *Alpinia officinarum* and their anticancer activity" Fitoterapia 79: 27–31.
6. Bendjeddou D., Lalaoui K., Satta D. (2003) "Immunostimulating activity of the hot water-soluble polysaccharide extracts of *Anacyclus pyrethrum*, *Alpinia galanga* and *Citrullus colocynthis*" Journal of Ethnopharmacology 88: 155-160.
7. Bernt J., Samuel P., Nicole G., Sanghyun C., Yuehong W., Scott G., Guido P., et al. (2008) "Anti-tuberculosis natural products in galangal (*Alpinia officinarum*) and ginger (*Zingiber officinale*)" Journal of Natural Products 71 (8):1489 –1508.
8. Bhowmick K., Chakraborti G., Gudi N.S., Moideen A.V.K., Shetty H.V. (2008) "Free radical and antioxidant status in rheumatoid arthritis" Indian Journal of Rheumatology 03(1): 08-12.
9. Borthakur M., Hazarika J., Singh R.S. (1999) "A protocol for micropropagation of *Alpinia galangal*" Plant Cell, Tissue and Organ Culture 55: 231–233.
10. Signals 12: 267-282.
11. Doug H., Chen S.X., Kadota S., Namba T. (1998) "A new antiplatelet diarylheptanoid from *Alpinia blepharocalyx*" Journal of Natural Products 61: 142–144.
12. Yoshikawa M., Matsuda H., Morikawa T., Managi H. (2003) "Antiallergic principles from *Alpinia galanga*: structural requirements of phenylpropanoids for inhibition of degranulation and release of TNF-α and IL-4 in RBL-2H3 cells" Bioorganic and Medicinal Chemistry Letters 13(19): 3197 – 3202.
13. Yoshikawa M., Matsuda H., Nakashima S., Oda Y., Nakamura S. (2004) "Melanogenesis inhibitors from the rhizomes of *Alpinia officinarum* in B16 melanoma cells" Bioorganic and Medicinal Chemistry 17: 6048–6053.
14. O. Oyedapo, A. Akinpelu *et al.*, Red blood cell membrane stabilizing potentials of extracts of Lantana camra and its fraction, International journal of plant physicology and biochemistry, October 2010,Vol 2(4),46-51