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Research Article

Pharmacological Screening of Combination of 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. *Gloriosa superba* 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 *Gloriosa superba* 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 *Gloriosa superba* 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.[3-6]

Materials and Methods[7-14]

Collection of plant: *Ipomoea carnea* The plant *Gloriosa superba* 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 *Gloriosa superba* all plant part 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 *Gloriosa superba* 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

***In-vitro* Anti-inflammatory activity of Plants Extracts[14]**

Ethanol extract of Gloriosa superba 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

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 *Gloriosa superba*

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 *Gloriosa superba 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 *Gloriosa superba* 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.

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	0.208±0.25 [a]	16.80
	2.00	0.182±0.22[a]	29.20
	4.00	0.147±0.28[b]	44.10
	6.00	0.123 ±0.42[c]	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	0.202±0.25 [a]	18.35
	2.00	0.175±0.12[a]	30.87
	4.00	0.143±0.23[a]	46.96
	6.00	0.110±0.44[c]	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.

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Conclusions

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.

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