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PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTI-INFLAMMATORY ACTIVITY OF THREE INDIGENOUS PLANTS BY RBC MEMBRANE STABILIZATION METHOD

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ABSTRACT

The systematic evaluation of the anti-inflammatory activity of indigenous plants used in wound healing will facilitate the development of efficient wound healing drugs**.** The present study was undertaken to evaluate the efficiency of anti-inflammatory activity of three plants used by traditional healers, viz:- *Hemigraphis colorata* (Blume) H.G. Hallier, *Leucas aspera* (Willd) Link and *Biophytum sensitivum* (L.) DC. Hexane, ethyl acetate, acetone and ethanol extracts of the plants, were subjected to evaluate Human RBC membrane stabilization potential, which influence the process of inflammation. Among the extracts screened the hexane and acetone extract of *Leucas aspera*, acetone extract of *Biophytum sensitivum* and ethanol extract of *Hemigraphis colorata* were exhibited significantly better activity than the other extracts and standard anti-inflammatory drug Diclofenac sodium. These observations will stimulate further research in the clinical application of the plants under study. The study proves the anti-inflammatory efficacy of the plants and they hold a good prospect for drug development against inflammatory diseases.

KEYWORDS: *Hemigraphis colorata* (Blume) H.G. Hallier, *Leucas aspera* (Willd) Link, *Biophytum sensitivum* (L.) DC, Anti-inflammatory activity and RBC membrane stabilization.

1. INTRODUCTION

Medicinal plants are the major sources of renewable and cheaper medicines. A large number of plants have been used for wound healing in traditional systems of medicine since ancient times. However prolonged inflammation may delay natural healing process, a good healing

agent should possess anti-inflammatory activity for the proper wound management.[1,2,3] The systematic evaluation of the anti-inflammatory activity of indigenous plants will facilitate the development of efficient wound healing agents**.** In this study, three indigenous medicinal plants used for wound healing by traditional healers of south India; viz:- *Hemigraphis colorata* (Blume) H.G. Hallier, *Leucas aspera* (Willd) Link and *Biophytum sensitivum* (L.) DC, were selected to evaluate their anti-inflammatory activity.

2. METHODOLOGY

2.1. Preparation of Plant Extracts

Fresh plants were collected from Aromatic and Medicinal Plant Research Station, Kerala Agricultural University Research Station, Odackali, Eranakulam, Kerala and were cultivated. The plant materials (leaves) were washed with tap water and were shade-dried at ambient temperature and then ground into powder using a grinder. The different extracts of *Hemigraphis colorata, Biophytum sensitivum* and *Leucas aspera* were prepared by soxhlet extraction method. 100g of air dried fine powder of each plant was extracted using soxhlet extraction apparatus, successively with solvents of increasing polarity for 72 hours. Solvents used were hexane, ethyl acetate, acetone and ethanol. All extracts were evaporated to dryness using rotary evaporator and the extraction yield for each extract was calculated.

2.2. Phytochemical Analysis

The hexane, ethyl acetate, acetone and ethanol extracts of *Hemigraphis colorata*, *Biophytum sensitivum* and *Leucas aspera* were subjected to a qualitative phytochemical screening, using standard methods.[4]

2.3. Anti-Inflammatory Assay (Human RBC Membrane Stabilization Method)

The extra cellular activity of lysosomal enzymes is said to be related to acute or chronic inflammation. The non-steroidal anti-inflammatory drugs act either by inhibiting these enzymes or by stabilizing the lysosomal membrane. Since the human RBC membrane is similar to lysosomal membrane components, the HRBC membrane stabilization has been used as a method to study the anti-inflammatory activity of drugs. The prevention of hypotonicity induced human RBC (HRBC) membrane lysis can be taken as a measure of anti-inflammatory activity of drugs.[5]

Blood was collected from healthy volunteers, who were not taken any drug for the past two weeks. Informed consent was obtained from the person. Approval of the Institutional ethics

committee was obtained. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline (0.85%, pH 7.2) and a 10% v/v suspension was made with isosaline. The assay mixture contained the plant extract (20mg/ml), 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Diclofenac sodium was used as the reference drug. Control was prepared without extract and drug control without HRBC suspension. Buffer and isosaline were taken as blank. All the assay mixtures were incubated at 37°C for 30 min and centrifuged. The haemoglobin content in the supernatant solution was estimated using spectrophotometer at 560nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water at 100%. The percentage of HRBC membrane stabilization or protection was calculated by using the formula,

$$\text{Human RBC membrane stabilization (\%)} = \frac{[100 - \text{Optical density of drug treated sample} - \text{Drug control}]}{\text{Optical density of control}} \times 100$$

2.4. Statistical Analysis

Experimental results were presented as the mean ± standard deviation (SD) of three parallel measurements. The statistical analysis was performed by one way ANOVA, followed by Duncan's test. Differences were considered statistically significant when p value were less than 0.05 using SPSS IBM-22.

3. RESULTS AND DISCUSSION

3.1. Preparation of Plant Extracts

Hexane, ethyl acetate, acetone and ethanol extracts of plants; *Hemigraphis colorata*, *Biophytum sensitivum* and *Leucas aspera* were prepared successively by soxhlet extraction method and the yield calculated was reported in Table 1. Acetone extract of *Leucas aspera* and ethyl acetate of *Biophytum sensitivum* and *Hemigraphis colorata* were found to have more yield. The yield of ethanol extract of *Hemigraphis colorata* was found to be 19.27g extract/100g dry plant powder, which is more than all other extracts. The solvents, acetone, ethyl acetate and ethanol generally could extract more organic components from plants.[6]

Table 1: Total yield of plant extracts (g per 100g dry plant).

Plant	Extract	Yield (g/100g)
Leucas aspera	Hexane	4.02
	Ethyl acetate	3.31
	Acetone	**6.92**
	Ethanol	0.96
Biophytum sensitivum	Hexane	4.61
	Ethyl acetate	**6.91**
	Acetone	0.92
	Ethanol	0.49
Hemigraphis colorata	Hexane	3.48
	Ethyl acetate	**15.45**
	Acetone	1.43
	Ethanol	**19.27**

3.2. Phytochemical analysis

Preliminary phytochemical screening of the hexane, ethyl acetate, acetone and ethanol extract of *Leucas aspera*, *Biophytum sensitivum* and *Hemigraphis colorata* revealed the presence of various phytochemicals like phenols, flavanoid, alkaloids, saponins and terpenoids and the result was summarized in Table.2. The ethanol and ethyl acetate extracts of all the plants contain more phytochemicals than the other extracts. The qualitative phytochemical analysis of hexane, ethyl acetate, acetone and ethanol extracts of *Leucas aspera*, *Biophytum sensitivum* and *Hemigraphis colorata* were supported by previous reports.[7,8,9] The result showed that the ethanol and ethyl acetate extracts of *Biophytum sensitivum* and *Hemigraphis colorata* contained more phytochemicals.

Table 2: Qualitative phytochemical analysis.

Sl. No.	Phytochemicals / Tests	Plant											
		Biophytum sensitivum				*Hemiographis colorata*				*Leucas aspera*			
		H	EA	A	E	H	EA	A	E	H	EA	A	E
1.	Carbohydrate a. Molisch'sTest b. Barfoed's Test c. Benedict's Test d. Fehling's Test	 - - - -	 + + + +	 - - - -	 + + + +	 - - - -	 + + + +	 - - - -	 + + + +	 - - - -	 - - - -	 + + + +	 + + + +
2.	Alkaloid Test a. Wagner's Test b. Mayer's Test	 - -	 - -	 + +	 - -	 - -	 - -	 + +	 - -	 - -	 - -	 + +	 + +
3.	Flavanoids (Alkaline reagent test)	-	+	-	+	-	+	-	+	-	+	-	+
4.	Protein (Biuret test)	-	-	+	-	-	-	+	-	-	-	-	+
5.	Phenols a. Lead acetate Test	 +	 +	 +	 +	 -	 +	 +	 +	 +	 +	 +	 +

	b. Ferric chloride test	+	+	+	+	-	+	+	+	+	+	+	+
6.	Oils (Spot test)	+	+	-	-	+	+	-	-	+	+	-	-
7.	Steroids(Salkowski's test)	+	-	+	+	+	-	-	+	+	-	-	+

Symbol (+) indicates presence and (-) indicates absence of phytochemicals. H-Hexane extract, EA-Ethyl acetate extract, A-Acetone extract, E-Ethanol extract.

3.3. Anti-inflammatory assay

In vitro anti-inflammatory assay by human RBC membrane stabilization method showed that all the extracts of the three plants under study, possessed good activity as shown in table.3. Hexane, ethyl acetate, acetone and ethanol extracts of *Leucas aspera* showed more than 50% of membrane stabilization. Among the four extracts, hexane and acetone extract has shown significantly higher percentage of membrane stabilization compared to standard anti-inflammatory drug Diclofenac. There are several studies regarding the anti-inflammatory effects of the plant.[10,11,12] Sadhu *et al* (2003) isolated phytocompounds from *Leucus aspera*, with prostaglandin inhibitory activity.[13] Similar observations made by others suggested *Leucas aspera* has better anti-inflammatory activity than acetylsalicylic acid in the carrageenin induced paw edema model.[14,15] In the present study, *in vitro* anti-inflammatory activity of the different extracts of *Biophytum sensitivum* was found to be more promising. The results showed that, all extracts possessed more than 50% of HRBC membrane stabilization. Among various extracts, the ethanol extract has shown highest membrane stabilization, which is significantly higher when compared to standard drug. This supports the previous reports of anti-inflammatory activity of alcoholic extract of *Biophytum sensitivum.*[16,17] In our study, ethanol extracts of *Hemigraphis colorata* has showed a good percentage of HRBC membrane stabilization than the other extracts (Fig.1). The results were incomparable with the previous studies.[18,19] As inflammation has been shown to delay healing and to increase scarring, plants having anti-inflammatory property could be useful as better wound healing agents.[20]

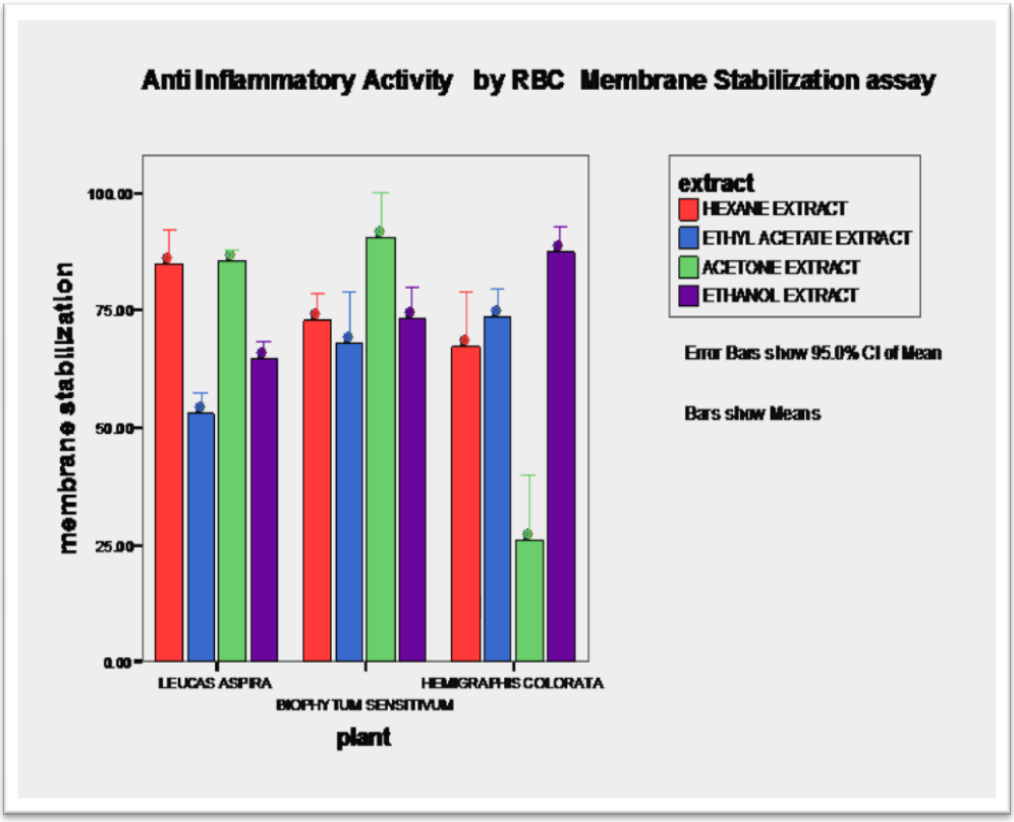

Fig. 1: Anti-inflammatory activity of various extracts of *Leucas aspera, Biophytum sensitivum* and *Hemigraphis colorata.*

Table 3: Anti-inflammatory activity of various extracts of *Leucas aspera, Biophytum sensitivum* and *Hemigraphis colorata* expressed as the percentage of RBC membrane stabilization.

Plant	Extract	RBC membrane stabilization (%)
Leucas aspera	Hexane	**84.74 ± 2.85[a]**
	Ethyl acetate	52.95 ± 1.88
	Acetone	**85.56 ± 0.84[a]**
	Ethanol	64.63 ± 1.45
Biophytum sensitivum	Hexane	72.98 ± 2.16
	Ethyl acetate	67.96 ± 4.38
	Acetone	**90.42 ± 3.87[a]**
	Ethanol	73.08 ± 2.80
Hemigraphis colorata	Hexane	67.32 ± 4.66
	Ethyl acetate	73.73 ± 2.35
	Acetone	25.80 ± 5.58
	Ethanol	**87.53 ± 2.13[a]**
Diclofenac sodium		76.02 ± 0.27

Values are expressed as Mean ± SD, P<0.05, a-significantly differ with standard Diclofenac

CONCLUSION

The current study adds more scientific evidence for the traditional use of *Hemigraphis colorata* (Blume) H.G. Hallier, *Leucas aspera* (Willd) Link and *Biophytum sensitivum* (L.) DC. Among the various plant extracts under study, the hexane and acetone extract of *Leucas aspera*, acetone extract of *Biophytum sensitivum* and ethanol extract of *Hemigraphis colorata* exhibited significantly better anti-inflammatory activity than the other extracts and standard anti-inflammatory drug Diclofenac sodium. Thus, further research on the identification of bioactive components responsible for activity, will pursuit new phytotherapeutics against inflammatory diseases.

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