

**ANTI-INFLAMMATORY ACTIVITY OF MORINDA UMBELLATA BY MEMBRANE STABILIZATION METHOD****J.N. DHARSANA<sup>1\*</sup> AND DR. SR. MOLLY MATHEW<sup>2</sup>**

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**ABSTRACT**

To evaluate the preliminary phytochemical and anti-inflammatory activities of various extracts of *Morinda umbellata*. *Morinda umbellata* (Family-Rubiaceae) commonly known as Ney-Valli in Malayalam, found in Southern Western Ghats. The present study aimed at preliminary evaluation of phytochemical and anti-inflammatory study of ethanol and aqueous extracts of *Morinda umbellata* by in vitro HRBC membrane stabilizing activity. Ethanol and aqueous extracts of *Morinda umbellata* were found to have significant anti-inflammatory activity. The ethanol and aqueous extract of *Morinda umbellata* showed potent anti-inflammatory activity when comparing with the standard drug Indomethacin, perhaps due to the presence of secondary metabolites like alkaloids, steroids, flavonoids, phenols and saponins.

**KEY WORDS:** *Morinda umbellata*, Rubiaceae, Anti-inflammatory, HRBC membrane stabilization.

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## INTRODUCTION

Inflammation is a complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants. It is a defensive mechanism of the organism to remove the injurious stimuli as well as to initiate the healing process for the tissue. In the absence of inflammation, wounds and infections would never heal and progressive destruction of the tissue would compromise the survival of the organism<sup>1</sup>. Many components are involved in the inflammation process to name a few are edema formation; leukocyte infiltration and granuloma formation are widely noticeable<sup>2</sup>. Inflammation that is chronic and uncontrolled becomes detrimental to tissues<sup>3</sup>. Since ancient times in various cultures worldwide, inflammatory disorders and related diseases have been treated with plants or plant derived formulations. The anti-inflammatory activity of several plant extracts and isolated compounds has already been scientifically demonstrated. *Morinda umbellata* is a climber of Rubiaceae family. In Malayalam the plant is known as Neyvalli. It is distributed in high ranges of Western Ghats. The tribal communities use this plant for diabetes, high blood pressure, muscle aches and pain.

## MATERIALS AND METHODS

### *Plant material*

Whole plants were collected from Wynad hills, Wynad district, Kerala, India. The taxonomical identification of the plant was done by Dr. N. Sasidharan, Scientist-F, Programme co-ordinator, FE& BC division, Kerala Forest Research Institute, Peechi, and Trissur. The voucher specimen was preserved in Academy of Pharmaceutical Sciences, Pariyaram Medical College, Kannur, Kerala.

### *Preparation of plant extract*

The collected whole plants were dried at room temperature, pulverized by a mechanical grinder, sieved through 40 mesh. About 200g of powdered materials were extracted with ethanol and water by successive solvent extraction method. The extracts were then concentrated

using vacuum evaporator and dried under reduced pressure.

### *Phytochemical screening*

The concentrated extracts were used for preliminary screening of various phytoconstituents viz; carbohydrate, amino acid, alkaloids, tannins and flavonoids were detected by usual methods prescribed in standard tests<sup>4,5,6</sup>.

### *In vitro anti-inflammatory activity by Human red blood cell (HRBC) membrane stabilizing activity<sup>7,8</sup>*

This method evaluates the membrane stabilizing activity of various agents on red blood cells against the osmotic pressure exerted by Alsever solution. Alsever solution is prepared by dissolving 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% of sodium chloride in distilled water followed by sterilization. Blood was collected from Pariyaram Medical College Blood Bank. The collected blood was mixed with equal volumes of Alsever's solution. The blood was centrifuged at 3000 rpm and the packed cells were washed with isotonic saline and 10% (v/v) suspension was made. The drug samples ranging from a concentration of 50 µg/ml-250 µg/ml were prepared by suspending the residue in hot water. The assay mixture contained the drug, 1 ml phosphate buffer, 2 ml hypotonic saline (0.25% w/v), 0.5 ml HRBC suspension. Indomethacin was used as the reference drug and 2 ml of distilled water as control. All the assay mixtures were incubated at 37°C for 30 minutes and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm. The percentage haemolysis was calculated by assuming the haemolysis produced in the presence of distilled water as 100%. Percentage of protection was calculated using the following equation.

Percentage Protection =  $1 - (\text{OD Sample} / \text{OD Control}) \times 100$

## RESULTS

### a) Phytochemical studies

Preliminary phytochemical analysis of ethanol and aqueous extracts showed the presence of secondary metabolites such as alkaloids, phenols, saponins, steroids, flavonoids. (Table 1)

### b) In vitro anti-inflammatory activity(HRBC membrane stabilization)

The membrane stabilizing activity of ethanol and aqueous extract of *Morinda umbellata* possess significant membrane stabilizing activity when compared with the control group. Inhibitory concentration (IC 50%) values ranged from 345.81 to 203.076 with values for Indomethacin (121.68µg/ml), ethanol extract (345.81µg/ml) and aqueous extract (203.076µg/ml). (Table 2)

**Table 1**  
**Phytochemical screening of whole plant of *Morinda umbellata*.**

Phytochemical constituents	Ethanol extract	Aqueous extract
Carbohydrates	—	—
Amino acids	—	—
Proteins	+	+
Fats	—	—
Steroids	+	+
Alkaloids	+	+
Saponins	—	+
Cardiac glycosides	—	+
Flavonoids	—	+
Tannins	+	+
Phenols	+	+
Starch	—	—
Quinone	—	+
Sugar	—	—

(+): Present

(-): Absent

**Table 2**  
**In vitro anti-inflammatory activity of ethanol and aqueous extract of *Morinda umbellata*.**

Sample	Concentration (µg)	Absorbance at 560nm	% Inhibition	EC <sub>50</sub> (µg/ml)
Control	-	0.912±0.012	-	
Indomethacin	50	0.593±0.006	34.98	121.68
	100	0.496±0.007	45.62	
	150	0.406±0.008	55.49	
	200	0.305±0.005	66.56	
	250	0.199±0.008	78.18	
Ethanol extract	50	0.726±0.004	20.04	345.81
	100	0.665±0.007	25.21	
	150	0.609±0.007	31.26	
	200	0.532±0.009	35.14	
	250	0.498±0.002	40.15	
Aqueous extract	50	0.676±0.009	25.68	203.076
	100	0.604±0.004	33.18	
	150	0.543±0.003	41.78	
	200	0.477±0.004	49.69	
	250	0.403±0.001	57.33	

Values are expressed as mean ± SEM, n=6 in each group

## DISCUSSION

Preliminary phytochemical analysis of *Morinda umbellata* indicated the presence of alkaloids, steroids, flavonoids, phenols, saponins<sup>9</sup>. Reported that all secondary metabolites could impart medicinal properties to of the plant and has specific healing properties, healthy action and non-toxic effects. It is well reported that phytochemicals are found to have a broad range of activities which may help in protection against chronic diseases<sup>10,11</sup>. The present study on preliminary phytochemical analysis provides a better understanding of this less explored medicinal plant. This analysis gave important information in the identification and authentication of the plant material. Lysosomes are single membrane structures that contain digestive enzymes. When certain white blood cells engulf bacteria, the bacteria are digested and destroyed by these lysosomal enzymes. Worn out cell parts and dead cells are also digested by these enzymes. This is a beneficial process and is necessary before tissue repair can begin. But it does not have a disadvantage in that lysosomal digestion contributes to inflammation in damaged tissues. An excessive inflammation can start a vicious cycle, actually a positive feedback mechanism that results in extensive tissue damage<sup>12</sup>. Various methods are employed to screen and study drugs, chemicals, herbal preparations that inhibit the inflammation. These techniques include uncoupling of oxidative phosphorylation (ATP biogenesis linked to respiration), inhibition of denaturation of protein, erythrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation<sup>13</sup>. Human Red Blood Cell stabilization against hypotonicity induced lysis was selected for the assessment of anti-inflammatory activity of *Morinda umbellata* due to its simplicity and reproducibility. HRBC membrane is similar to the lysosomal membrane. During inflammation, histamine from damaged tissues makes capillaries more permeable and the lysosomes of damaged cells release their enzymes which help breakdown damaged tissue but may also

cause destruction of nearby healthy tissue. Some of the NSAIDs and glucocorticoids stabilize lysosomes in tissue cells and there by prevent the release of lysosomal enzymes into the cytoplasm of the cells, thus preventing deterioration from this source<sup>14</sup>. Stabilization of lysosomal membrane therefore can control inflammatory response and therefore stabilization of human red blood cell (HRBC) from hypotonicity induced lysis can be correlated with the anti-inflammatory potential of a drug. Some of the Indian medicinal plants like *Cassia grandis* Linn<sup>15</sup>, *Ficus carica*<sup>16</sup>, *Abroma augusta* Linn<sup>17</sup> have been screened for their anti-inflammatory activity by various researchers so far. The assay method involves incubation of RBCs into a hypotonic solution (less than 282 mOsm/L), so that water will diffuse into the cell, causing it to swell; water will continue to diffuse into the cell, resulting in lysis of the cell. In the present study various extracts of *Morinda umbellata* possess significant stabilizing of HRBC, the probable mechanism of protection of hypotonicity induced lysis is shrinking of the cell membrane and involves processes that prevent the migration of these intracellular components outside the cell. It has been shown that cell deformability and cell volumes of erythrocytes are closely related to their intracellular content of calcium<sup>18</sup>. Thus the membrane stabilization effect by of these agents may be due to, alteration of the influx of calcium into the erythrocytes. The precise mechanism for these effects remains to be elucidated.

## CONCLUSION

Present study reveals that *Morinda umbellata whole plant* could be a useful resource as biotherapeutic agents. In vitro results indicates that it possess anti-inflammatory activity. So these efforts could open up the possibility of finding new clinically useful biotherapeutic agents.

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