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**EVALUATION OF *IN-VITRO* ANTI-INFLAMMATORY ACTIVITY
OF HERBAL PREPARATION, A COMBINATION OF FOUR
MEDICINAL PLANTS**

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ABSTRACT

Inflammation is initiated as a healing process by the tissue in response to an injury by pathogens, irritants or cell damage. Anti-inflammatory drugs do have their side-effects. The focus of research studies is upon the use of medicinal plants as anti-inflammatory agents over the past years. In the present study, 80% alcoholic extract of leaves of Aloe vera, Bacopa monnieri, Moringa oleifera and rhizome of Zingiber officinale was used to prepare a Herbal Preparation (HP-4 of 100 mg /10ml methanol concentration). Different concentrations of HP-4 was used to study the in-vitro anti-inflammatory activities in terms of effect of hypotonic solution –induced haemolysis on RBC membrane stabilization and effect of inhibition of protein denaturation activity. Acetylsalicylic acid was used as reference drug which showed comparable anti-inflammatory activity as compared to control sample in which no drug was used. It was found that HP-4 has dose dependent RBC membrane stabilization and inhibition of protein denaturation activity.

Key Words: *Inflammation, In-Vitro Activity, Herbal Preparation, Membrane Stabilization, Protein Denaturation*

INTRODUCTION

Inflammation is a normal protective response to tissue injury and it involves a complex array of enzyme activation, mediator release, fluid extravasations, cell migration, tissue breakdown and repair (Vane *et al.*, 1995). It is a complex process, which is frequently associated with pain and involves occurrences such as: the increase in vascular permeability, increase of protein denaturation and membrane alterations (Umapathy *et al.*, 2010).

Harmful stimuli including pathogens, irritants or damaged cells initiate response of vascular tissue as inflammation. Inflammation is a protective attempt by the organism to remove injurious stimuli as well as initiate the healing process for the tissue (Denko, 1992). However, if inflammation is not treated it leads to onset of diseases like vasomotor rhinorrhoea, rheumatoid arthritis and atherosclerosis (Henson *et al.*, 1989).

In appreciating the inflammatory process, it is important to understand the role of chemical mediators. These are substances that tend to direct the inflammatory response. These inflammatory mediators come from plasma proteins or cells including mast cells, platelets, neutrophils and monocytes /macrophages. They are triggered by bacterial products or host proteins. Chemical mediators bind to specific receptors vascular permeability, neutrophil chemotaxis, stimulate smooth muscle contraction, have direct enzymatic activity, induce pain or mediate oxidative damage. Most mediators are short - lived but cause harmful effects. Examples of chemical mediators include vasoactive amines (histamine, serotonin), arachidonic acids (prostaglandins, leukotrienes) and cytokines (tumour necrosis factor and interleukin -1) Smith *et al.*, (2004).

It is believed that current drugs available such as opioids and non –steroidal anti-inflammatory drugs (NSAIDs) are not useful in all cases of inflammatory disorders, because of their side effects and potency (Ahmadiani *et al.*, 1998). As a result, a search for other alternatives seems necessary and beneficial. The study of plants that have been used traditionally for curing

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inflammation is still fruitful and logical research strategy in the source of new anti-inflammatory drugs (Kumarappan *et al.*, 2006).

Medicinal plants have a wide variety of chemicals from which novel anti-inflammatory agents can be discovered. Research on the biological activities of plants during the past two centuries has yielded compounds for the development of modern drugs (Arivazhagan *et al.*, 2000).

The botanical name of Aloe vera is *Aloe barbadensis* miller. It is commonly known as 'Khorpad' in Marathi. It belongs to Asphodelaceae (Liliaceae) family and is shrubby or arborescent, perennial, xerophytic, succulent pea-green colour plant. It grows mainly in the dry regions of Africa, Asia, Europe and America. In India, it is found in Rajasthan, Andhra Pradesh, Gujarat, Maharashtra and Tamil nadu (Surjushe *et al.*, 2010). It inhibits the cyclooxygenase pathway and reduces prostaglandins E2 production from arachidonic acid. Recently, the novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts (Hutter *et al.*, 1996).

Bacopa monnieri L. (Family Scrophulariaceae) commonly known as 'brahmi' is a creeping, glabrous, succulent herb, rooting at nodes, distributes throughout India in all plain districts. The plant is reported to show sedative, antiepileptic, vasoconstrictor and anti-inflammatory activity (Anonymous, 1998). It has been reported that the plant contains tetra cyclic triterpenoid saponins, bacosides A and B, bacosaponin, alkaloids viz. herpestine and brahmine and flavonoids (Anonymous, 1998 and Kirtikar *et al.*, 1994).

Moringa oleifera Lam. (horseradish tree, drumstick tree) is the most widely cultivated species of a monogeneric family, the Moringaceae. All parts of the *Moringa* tree are edible and have long been consumed by humans (Fuglie 1999).

Various parts of *Moringa* acts as cardiac and circulatory stimulants, possess antitumour, antipyretic, antiepileptic, anti-ulcer, anti-spasmodic, diuretic, anti-hypertensive, cholesterol lowering, antioxidant, anti-diabetic and anti-inflammatory (Nepolean *et al.*, 2009).

Zingiber officinale R., commonly known as 'ginger' (Zingiberaceae) is cultivated commercially in India, China, South East Asia, West Indies, Mexico and other parts of the world. It is consumed worldwide as a spice and flavouring agent and attributed to have many medicinal properties. *The British Herbal Compendium* reported its actions as a carminative, anti-emetic, spasmolytic, peripheral circulatory stimulant and anti-inflammatory (Bradley, 1992). In *Ayurveda*, ginger exerts its ameliorative effects related to inhibition of prostaglandins and leukotriene biosynthesis (Kiuchi *et al.*, 1992).

The four selected medicinal plants i.e. leaves of *Aloe vera*, *Bacopa monnieri*, *Moringa oleifera* and rhizome of *Zingiber officinale* were used to prepare a Herbal Preparation (HP-4). The aim of the present study was to evaluate the *in-vitro* anti-inflammatory activity in terms of RBC membrane stabilization and protein denaturation activity.

MATERIALS AND METHODS

Plant Material: Leaves of *Aloe vera*, *Bacopa monnieri*, *Moringa oleifera* and rhizome of *Zingiber officinale* were collected from Loni and adjoining areas, Maharashtra. The individual plants were identified and authenticated by a Professor of Botany, Loni. The four medicinal plants were shade dried and powdered. The selected parts of medicinal plants were extracted in 80% ethanol by hot extraction in Soxhlet apparatus till colourless solvent was obtained. Extracts obtained were allowed to dry till constant weight was obtained. The % yield of extract of each plant part was *Aloe vera* 28.62%, *Bacopa monnieri* 16.18%, *Moringa oleifera* 14.90% and *Zingiber officinale* 12.69%.

Herbal Preparation (HP-4): Plant mixture was prepared by mixing each extract 25mg dissolved in 10ml methanol (that is 100 mg /10ml) and boiled. It was centrifuged at 2500 rpm for 10

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minutes. The supernatant thus obtained was named as Herbal Preparation (HP-4) and used in experiments.

Phytochemical Analysis: The herbal preparation was subjected to preliminary phytochemical studies using standard procedures to detect the phytochemicals present.

Estimation of Total Phenolic Compounds: Total phenolic content was determined by the Folin Ciocalteu method (Folin et al., 1927). To 0.5ml of 1-5 mg/ml of herbal preparation made up with 0.5ml of distilled water, 0.5 ml of Folin Ciocalteu reagent was added and gently mixed. After 2 minutes 0.5ml of 100mg/ml sodium carbonate was added. The contents were mixed and allowed to stand for 2 hours. The optical density of the blue coloured samples was measured at 765 nm spectrophotometrically. Standard gallic acid of concentration 100-500 microgram/ml was used. The concentration of total phenolics is expressed as milligram of gallic acid /g of mixture. All determinations were carried out in triplicate.

Estimation of flavonoids: The method used (Chang et al., 2002) with slight modifications was followed for estimation of flavonoids. 0.5ml of concentration 100-500 µg/ml of herbal preparation was mixed with 1ml aluminium trichloride in ethanol (20g/l) and diluted with ethanol to 25 ml. The absorbance at 415 nm was read after 40 minutes at 37 °C. Rutin of concentration 0.5mg/ml, 1.0mg/ml, 1.5mg/ml, 2.0mg/ml and 2.5mg/ml was used as a reference compound and absorbance was measured under the same conditions. All determinations were carried in triplicate. The amount of flavonoids in herbal preparation was calculated as milligram of rutin/g of mixture.

Estimations of flavonols: The content of flavonols was determined (Yermakov et al., 1987) with slight modifications. 0.05 ml of various concentration (100-500 µg) was treated with 1ml of 2% aluminium trichloride in ethanol and 1ml of 5% sodium acetate. The absorption at 400nm was read after 2.5 hours at 37 °C. The same procedure was carried out for 2ml of reference compound rutin for concentration 0.2mg/ml, 0.4mg/ml, 0.6mg/ml, 0.8mg/ml and 1.0mg/ml. All determinations were carried out in triplicate. The content of flavonols was calculated in terms of milligram of rutin /g of mixture.

Drug used as Standard: Acetylsalicylic acid available in the commercial name of Ecosprin[®]-75 marketed by USV Limited, Mumbai, Maharashtra was used as a source of Acetylsalicylic acid.

Human Blood: The blood was collected from a healthy human volunteer who had not taken any NSAIDs for 2 weeks prior to the experiment and collected in heparinized vacutainer. The blood was washed three times with 0.9% saline and centrifuged simultaneously for 10 minutes at 3000 rpm. The packed cells were washed with 0.9% saline and a 40% v/v suspension made using isotonic phosphate buffer which was composed of 154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4 used as Stock erythrocyte or RBC suspension.

Hypotonic solution –induced haemolysis or membrane stabilizing activity: This test was done according to the method described (Shinde et al., 1999) with slight modifications. The test sample consisted of stock erythrocyte (RBC) suspension 0.030ml mixed with 5ml of hypotonic solution (154mM NaCl in 10mM Sodium Phosphate Buffer at pH 7.4) containing Herbal Preparation (HP-4) ranging from concentration 100-500 µg/ml. The control sample consisted of 0.030ml RBC suspension mixed with hypotonic buffered solution alone. The standard drug acetylsalicylic was treated similar to test at 100 and 200 µg/ml concentrations. The experiment was carried out in triplicate. The mixtures were incubated at 10 minutes at room temperature, centrifuged for 10 minutes at 3000rpm and absorbance of the supernatant was measured spectrophotometrically at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated by following equation.

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

Where:

A₁ = Absorbance of hypotonic buffered solution alone

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A_2 = Absorbance of test /standard sample in hypotonic solution.

Effect on Protein Denaturation: Protein denaturation was performed as described (Elias et al., 1988) with slight modifications. Test solution consisting of 1ml of different concentrations of Herbal Preparation (HP-4) ranging from 100-500 $\mu\text{g/ml}$ or standard acetylsalicylic acid 100 and 200 $\mu\text{g/ml}$ was mixed with 1ml of egg albumin solution (1mM) and incubated at $27 \pm 1^\circ\text{C}$ for 15 minutes. Denaturation was induced by keeping the reaction mixture at 70°C in a water bath for 10 minutes. After cooling the turbidity was measured spectrophotometrically at 660 nm. Percentage inhibition of denaturation was calculated from control where no drug was added. Each experiment was done in triplicate and the average was taken.

Statistical analysis: Data was represented as mean \pm SD, which was statistically analyzed by Student's t-test and $p < 0.001$ vs. control were considered to be significant.

RESULTS

Table 1: *In-vitro* anti-inflammatory effect of HP-4 evaluated by hypotonic solution – haemolysis and protein denaturation methods.

S no	Treatment	Conc "	% Inhibition	
		($\mu\text{g/ml}$)	Of haemolysis	Protein denaturation
1.	HP-4	100	16.52 ± 0.022	60.16 ± 0.002
		200	$18.26 \pm 0.023^*$	$61.79 \pm 0.003^*$
		300	$27.54 \pm 0.028^*$	$64.23 \pm 0.014^*$
		400	$28.99 \pm 0.030^*$	$66.67 \pm 0.002^*$
		500	$30.72 \pm 0.030^*$	$67.48 \pm 0.018^*$
2.	Std			
	Acetyl-salicylic	100	32.75 ± 0.080	31.70 ± 0.028
		200	$39.13 \pm 0.102^*$	$34.96 \pm 0.028^*$

Values are mean \pm SD, $n=3$, *Significant values, $p < 0.001$, using Student's t-test.

The phytochemical analysis of HP-4 showed that it is rich in polyphenols. The polyphenol content was 29.53 ± 0.42 mg/g of gallic acid equivalent. The amount of flavonoids in HP-4 in rutin equivalent was 24.05 ± 0.57 mg/g and the content of flavonols was 17.90 ± 0.24 mg/g. In the study of membrane stabilization activity of HP-4 at concentration range of 100-500 $\mu\text{g/ml}$ protected significantly in a concentration dependent manner the erythrocyte membrane against lysis induced by hypotonic solution. Acetylsalicylic acid in the concentration of 100-200 $\mu\text{g/ml}$ used as standard also offered significant ($p < 0.001$) protection of RBC's membrane against damaging effect induced by hypotonic solution. The membrane stabilization action and inhibitory effect of different concentration of HP-4 is presented in Table 1. The inhibitory effect of different concentrations of Herbal Preparation HP-4 on protein denaturation as shown in table form. HP-4 (100-500 $\mu\text{g/ml}$) and acetylsalicylic acid (100-200 $\mu\text{g/ml}$) showed significant inhibition of denaturation of egg albumin in concentration dependent manner. Both membrane stabilization activity and effect on protein denaturation contribute to the *in-vitro* anti-inflammatory activity of the Herbal Preparation (HP-4) used in our study.

DISCUSSION

The Herbal Preparation HP-4 was initially subjected to erythrocyte (RBC) membrane stabilization induced haemolysis by hypotonic solution. The erythrocyte membrane resembles to

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lysosomal membrane and as such the erythrocyte could be extrapolated to the stabilization of lysosomal membrane (Omale et al., 2008). The vitality of cells depends on the integrity of their membranes, exposure of RBC's to injurious substances such as hypotonic medium results in lysis of its membrane accompanied by haemolysis and oxidation of haemoglobin (Augusto et al., 1982 and Ferrali et al., 1992). An injury to RBC membrane will further render the cell more susceptible to secondary damage through free radical induced lipid peroxidation. (Augusto et al., 1982 and Ferrali et al., 1992).

It is therefore expected that phytochemicals present and their synergistic action as in HP-4 with membrane –stabilizing properties, should offer significant protection of the cell membrane against injurious substances (Maxwell. 1995 and Liu et al., 1992). Compounds with membrane –stabilizing properties are well known for their ability to interfere with release of phospholipases that trigger the formation of inflammatory mediators (Aitadafouri et al., 1996).

Studies on *Phyllanthus amarus* for the stabilizing activity could lead to an increase in the surface area to volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane proteins according to Chopade et al (2012); also supported by similar studies on *Phyllanthus amarus* by Mahat et al., (2007) and Iranboye et al., (2011).

The mechanism may be operative in our studies for Herbal Preparation HP-4.

Moreover, it has also been shown that the deformability and cell volume of erythrocytes is closely related to the intracellular content of calcium (Shinde et al., 1999 and Gambhire et al., 2009).

A possible explanation for the stabilizing activity of the extractives is due to an increase in surface area /volume ratio of the cells which could be brought about by an expansion of membrane or shrinkage of the cell and an interaction with membrane protein (Abe et al., (1991).

Chopade et al .,(2012) reported that the cytoprotective effect on erythrocyte membrane maybe due to the ability of the test extract *Phyllanthus amarus* to alter the influx of calcium. The investigation also suggested that the membrane stabilizing activity of *Phyllanthus amarus* maybe playing a significant role in its anti-inflammatory activity.

Studies on *in-vitro* anti-inflammatory activity of leaf extracts of *Basella alba*. Linn Var alba by Kumar V et al., (2011) exhibited membrane stabilization effect by inhibiting hypotonicity induced lysis of erythrocyte membrane. Stabilization of lysosomal membrane is important in limiting the inflammation response by preventing the release of lysosomal constituents of activated neutrophil such as bactericidal enzymes and proteases, which cause further tissue inflammation and damage upon extracellular release.

Similar to studies by Sadique et al.,(1989) and Olugbenga et al ., (2005); Malomo et al .,(2011) suggested that the high membrane stabilizing activity of the extract of *Celosia argentea* which has potential to protect the erythrocyte membrane from free radical damage. The membrane stabilizing activity of the extract may be due to the presence of flavonoids, alkaloids, tannins and or saponins present in HP-4.

Our Herbal Preparation HP-4 was then subjected to prevent protein denaturation effect on egg albumin solution. Several anti-inflammatory drugs have shown dose dependent ability to inhibit thermally induced protein denaturation (Grant et al., 1970). Denaturation of proteins is a well documented cause of inflammation in conditions like rheumatoid arthritis (Mizushima et al., 1964) .Thus protection against protein denaturation, which was the main mechanism of action of NSAIDS postulated by Mizushima et al., (1964) before the discovery of their inhibitory effect on cyclooxygenase by Vane., (1971) may play an important role in the antirheumatic activity of NSAIDS.

Gambhire et al., (2009) reported that methanol extract of *Murraya koenigi* leaves produces significant anti-inflammatory activities in dose dependent manner in membrane stabilization and inhibition of protein denaturation. Umapathy et al., (2010) confirmed that aqueous extract of

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Albuca setosa possess membrane stabilization properties, limiting protein denaturation process and white blood cell anti-migration properties. Ability of *Phyllanthus amarus* extract to bring down thermal denaturation of proteins is possibly contributed for its anti-inflammatory activity by Chopade *et al.*, (2012).

Therefore the Herbal Preparation (HP-4) consisting of four selected medicinal plants eventually leads to effective RBC membrane stabilization and inhibition of protein denaturation both contributing to it's *in-vitro* anti-inflammatory activity. This study also suggests the therapeutic role of HP-4 as an anti-inflammatory agent.

REFERENCES

- Abe H, Katada K, Orita M and Nishkibe M (1991). Effects of calcium antagonists on the erythrocyte membrane. *Journal of Pharmacy Pharmacology* **41**(1) 22-26.
- Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejad M and Saremi S (1998). Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. *Journal of Ethnopharmacology* **61**(2) 229-232.
- Aitadafouri M, Mounnnieri C, Heyman SF, Binistic C, Bon C and Godhold J (1996). 4-Alkoxybenzamides as new potent phospholipase A₂ inhibitors. *Biochemical Pharmacology* **51** (5) 737-742.
- Anonymous (1998). Indian Herbal Pharmacopoeia Vol I IDMA Mumbai 30-37.
- Arivazhagan S, Balasenthi S and Nagini S (2000). Antioxidant and anti-inflammatory activities of *Mallotus oppositifolium*. *Journal of Phytotherapy Research* **14** (4) 291-293.
- Augusto O, Kunze KL and Montellano PR (1982). N phenylprotoporphyrin formation in the haemoglobin phenylhydrazine reaction. *Journal of Biological Chemistry* **257** (11) 6231-6241.
- Bradley PR (1992). **British Herbal Compendium**, Bournemouth (U K) (British Herbal Medicine Association U K) **1** 190.
- Chang C, Yang M and Wen H (2002). Estimation of total flavonoids content in propolis by two complementary colorimetric methods. *Journal of Food & Drug Analysis* **10** (3) 178-182.
- Chopade AR, Sontakke PM and Sayyad FJ (2012). Membrane stabilizing activity and protein denaturation: A possible mechanism of action for the anti-inflammatory activity of *Phyllanthus amarus*. *Journal of Karad Institute of Medical Sciences University* **1** (1) 67-72.
- Denko C W (1992). A role of neuropeptides in inflammation. In: Whicher J T, Evans S W, eds. *Biochemistry in Inflammation*, ed. London: Kluwer Publisher, 177-181.
- Elias G and Rao M N (1988). Inhibition of albumin denaturation and anti-inflammatory activity of dehydrozingerone and its analogs. *Indian Journal of Experimental Biology* **26** (10) 540-542.
- Ferrali M, Signorni C, Ciccoli L and Comporti M (1992). Iron release and membrane damage in erythrocytes exposed to oxidizing agents phenylhydrazine, divicine and isouramil. *Biochemical Journal* **285** (1) 295-301.
- Folin O and Ciocalteau V (1927). On tyrosine and tryptophan determinations in proteins. *Journal of Biological Chemistry* **27**(2) 627-650.
- Fuglie LJ (1999). *The Miracle Tree: Moringa oleifera: Natural Nutrition for the Tropics*. Church World Service, **Dakar** **1** (1) 68.
- Gambhire M, Juvekar A and Wankhede S (2009) "a". Evaluation of anti-inflammatory activity of methanol extract of *Barleria cristata* leaves by *in vivo* and *in vitro* methods. *The Internet Journal of Pharmacology* **7**(1) 1-4.
- Gambhire MN, Juvekar AR and Sakat SS (2009) "b". Evaluation of anti-inflammatory activity of methanol extract of *Murraya koenigi* leaves by *in vivo* and *in vitro* methods. *Pharmacologyonline* **1**(10) 1072-1094.

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Grant NH, Album HE and Kryzanasuskas C (1970).Stabilization of serum albumin by anti-inflammatory drugs. *Biochemical Pharmacology* **19**(3) 715-722.

Henson PM and Murphy RC (1989). Mediators of the inflammatory process .Amsterdam Elsevier 404.

Hutter JA, Salmon M, Stavinoha WB, Satsangi N, Williams RF and Streeper RT (1996). Anti- inflammatory C-glucosyl chromone from *Aloe barbadensis*.*Journal of Natural Products* **59** (5) 541-543.

Iranboye BO, Owoyele VB, Kelani OR and Olaleye SB (2011).Analgesic activity of aqueous leaf extracts of *Phyllanthus amarus*. *African Journal of Medicine and Medical Science* **40** (1) 47-50.

Kirtikar KR and Basu BD (1994).Indian medicinal plants **Vol I**, Bishen Singh Mahendrapal Singh, Dehradun 1816-1817.

Kiuchi F, Iwakami S, Shibuga M, Hanaoka F and Sankawa U (1992).Inhibition of prostaglandins and leukotriene biosynthesis by gingerols and diaryl heptanoids.*Chemical and Pharmaceutical Bulletin* **40** (2) 387-391

Kumarappan CT, Chandra R and Mandal SC (2006).Anti-inflammatory activity of *Ichnocarpus frutescens*. *Pharmacologyonline* **3** (2) 201-206.

Kumar V, Bhat ZA, Kumar D, Bohra P and Sheela S (2011).*In –vitro* anti –inflammatory activity of leaf extracts of *Basella alba* Linn.Var Alba .*International Journal of Drug Development and Research* **3** (3)176-179.

Liu GT, Zhang TM, Wang BE and Wang YH (1992).Protective action of seven natural phenolic compounds against peroxidative damage to biomembranes. *Biochemical Pharmacology* **43** (2) 147-152.

Mahat MA and Patil BM (2007).Evaluation of anti-inflammatory activity of methanol extracts of *Phyllanthus amarus* in experimental animal models. *Indian Journal of Pharmaceutical Science* **9** (1) 33-36.

Malomo SO, Ore A and Yakubo MT (2011).*In vitro* and *in vivo* antioxidant activity of the aqueous extract of *Celosia argentea* leaves. *Indian Journal of Pharmacology* **43**(3) 278-285.

Maxwell SRJ (1995).Prospects for the use of antioxidant therapies. *Drugs* **49** (3) 345-361.

Mizushima Y and Kobayashi M (1968).Interaction of anti-inflammatory drugs with serum proteins, especially with same biologically active proteins. *Journal of Pharmacy and Pharmacology* **20** (1)169-173.

Nepolean P, Anitha J and Emilin-Renitta R (2009). Isolation, analysis and identification of phytochemicals of antimicrobial activity of *Moringa oleifera* Lam. *Current Biotica* **3**(1) 33-39.

Olugbenga M, Fafunso MA and Makinde JM (2005).Membrane stabilizing activity: A possible mechanism of action of anti-inflammatory property of *Gongronema latifolium* leaves. *International Journal of Biomedical and Health Science* **1** (1)1-14.

Omale J and Okafor PN (2008).Comparative antioxidant capacity and cytotoxicity of the leaf and stem of *Cissus multistriata*. *African Journal of Biotechnology* **7**(17) 3129-3133.

Sadique J, Al –Raqobah WA, Bugarith ME and El-Gindy AR (1989).The bioactivity of certain medicinal plants on the stabilization of RBC membrane system. *Fitoterapia* **60** (6) 525-532.

Shinde U A, Phadke AS, Nair AM, Mugantiwar AA, Dikshit VJ and Saraf VO (1999).Membrane stabilizing activity –a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia* **70** (3) 251-257.

Smith GR and Sortins Missailidis (2004).Cancer, inflammation and the AT₁ and AT₂ receptors. *Journal of Inflammation* **1** (3) , 10.1186/1476-9255-1-3.

Surjushe A, Vasani R and Saple DG (2008).*Aloe vera*: A short review. *Indian Journal of Dermatology* **53** (2) 163-165.

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Umapathy E, Ndebia EJ , Meeme A, Adam B, Menziura P, Nkeh-Chungag BN and Iputo JE(2010).An experimental evaluation of *Albuca setosa* aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *Journal of Medicinal Plant Research* **4** (5) 789-795.

Vane JR (1971). Inhibition of prostaglandins synthesis as a mechanism of action for aspirin like drugs. *Nature* **231**(2) 232-235.

Vane JR and Botting RM (1995).New insights into the mode of action of anti-inflammatory drugs. *Inflammation Research* **44** (1) 1-10.

Yermakov AI, Arasmov VV and Yarosh NP (1987). Methods of Biochemical analysis of Plants. Agropromizdat Leningrad (In Russian).