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IN VITRO EVALUATION OF ANTI INFLAMMATORY ACTIVITY OF DIFFERENT FRACTIONS OF ALOE VERA BARBADENSIS MILLER ROOT

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Abstract: The present study was undertaken to evaluate the efficiency of various extracts of Aloe vera *Barbadensis Miller* root for anti-inflammatory activity by simple, reliable less toxic less time consuming, activity checked under the HRBC membrane stabilization method. Since HRBC membrane is similar lysosomal membrane which influence in the process of inflammation. Water ethanol, extracts of aloe vera root was subjected to check the stabilization of HRBC membrane to predict the anti-inflammatory activity.
Keywords: Aloe Vera, Inflammation, HRBC

Introduction: The Ayurveda system of medicine is prevalent in India since the Vedic period and as early as the down of human civilization. Though Ayurveda has undergone many changes in the cause of its long history, it still remains the mainstay of medical relief to a larger section of population of the nation. (1)
The Indian history of medicinal plants is dated back to 3500BC the curative properties of plants have been mentioned in the sukts riged and atharvaveda. Ayurveda has also described good number of plants medicine of plants with their therapeutic properties. (3) The ancient well known Tretise in Ayurveda, the Charak Samhita and Susrut samhita were written by Charak and Susrut respectively.
Medicinal plants has been founded by naturally plant represent the medicinal activity and other compounds. Since ancient times, medicinal plants are used to curve several types of health problems. Systemic analyses of these plants provide a variety of bioactive molecules for the development of newer pharmaceutical products. Recently, there is a growing interest in the pharmacological evaluation of various used in different traditional known plant have been extensively studied by advanced scientific technique and reported for various medicinal properties. Anti-inflammatory, antifungal, anti-cancer, Ant diabetic activity, anthelmintic, antibacterial activity, antifungal activity, hepatoprotective activity, antioxidant activity, larvicidal activity etc. (5)

Inflammation: Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. Inflammation is a protective immune vascular response that involves immune cells, blood vessels, and molecular mediators. The purpose of inflammation is to eliminate the initial cause of cell injury, clear out necrotic cells and tissue damaged from the inflammatory process, and to initiate tissue repair. Inflammation is a generic response, and therefore it is considered as a mechanism of initiate immunity, as compared to adaptive immunity, which is specific for each pathogen. (13)

Materials and Methods
Plant Profile: Aloe Vera *Barbadensis Miller* Taxonomy

Kingdom	-	Plantae
Order	-	Asparagales
Division	-	Spermatophyte
Subdivision	-	Angiospermate
Class	-	Monocotyledoneae
Genus	-	Aloe
Species	-	Barbadensis miller
Family	-	Asphodelaceae

Description: Aloe-Vera *barbadensis miller* is a very effective and important herbal plantin manyother plants, it gives so many medicinal activities and pharmacological effects for human beings and animal. Aloe-Vera also can be used for medicinal application in different system of our cultures. Aloe Vera acts an antimicrobial agent that removes and inhibit the growth and development of microorganism (bacteria), fungi, protozoan etc. the

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antimicrobial drugs are removes and supressed the microbes or protect the growth and development of (micro-biostatic). Various parts of this plant were useful in curing a wide range of health related issue. This plant synthesizes a vast array of secondary metabolites that are important for medicine.
Aloe Vera has been used by mankind for thousands of years in folk medicine for therapeutic properties especially on skin. This plant is one of the oldest known and its first documented use by humans dates back to an Egyptian papyrus from 3500BC. The Greek philosopher Aristotle wrote about the beneficial medicinal effect of aloe Vera, while references are also found throughout the bible. The ancient Greeks, romans, Chinese and Indians used it.[16]
By the early 1800s aloe Vera was served as laxative in the United States. Moreover, Morden clinical use began in the 1930s with reports of successful treatment of x-ray and radium burns aloe Vera drives its name from the Arabic word alloeh which means shining bitter substance because of the bitter liquid found in the leaves and Vera which means' true' in the Latin the species of was first described by carl Linnaeus in 1753whu suggested the following classification; kingdom plant.

The natural range of aloe Vera is nuclear as the species has been widely cultivated throughout the world, rather originating in Africa it is grown in most subtropical and tropical locations including South Africa .and Latin America. Then it was introduced to china. India and various parts of southern Europe in the 17th century aloe vera is a cactus-like plant, although is related to the onion, garlic and asparagus it is stem less with triangular, fleshy leaves ranging in colour from grey-green to bright green and in the margin of the leaves has small white teeth. The leaves composed of three layers an inner gel a yellow sap and the outer thick layer of 15-20 cells called as rind. Aloe leaves have long been used for medical and cosmetic purposes as well in health
Early in 1941 was reported that the leaf pulp of aloe vera containing 98.5%water and its alcoholic-insoluble portion was a mucilage containing uronic acid, fructose, hydrolysable sugars and enzymes nowadays, it's also known as gel.[9]

Chemical Constituents:

1. Mono and polysaccharides
2. Amino acid
3. Enzymes
4. Vitamins
5. Minerals
6. Sugars
7. Saponin
8. Salicylic acid hormones

Habitat and Ecology: Aloe Vera *Barbadensis miller* has very short stem it may be considered stem-less. It is succulent plant that can grow up to 80-100cm tall. The leaves are thick and fleshy, with serrated margin, the color ranges from green to gray green

Fig no.1 of root and leaves of aloe vera


Fig no.2 of root and leaves of aloe vera

Collection and authentication of plant.
The fresh plant Aloe-Vera root was collected from collected from FRI (Forest Research Institute) Dehradun.

Determination of ash value
(Pharmacopoeia standards for Ayurveda formulation, 1987, Quality control methods for medicinal plant materials, 1998).

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The ash of any organic material is composed of their non-volatile inorganic components. Controlled incineration of crude drug results in an ash residue consisting of inorganic material (metallic salts and silica). This value varies within fairly wide limits and is, therefore, an important parameter for the purpose of evaluation of crude drugs. The ash value can be determined by three different methods to measure the total ash, the acid insoluble ash and the water soluble ash.

Determination of total ash
Total ash is designed to measure the total amount of material produced after complete incineration of the ground drug at as low as temperature as possible (about 450^{o} C) to remove all the carbons. At higher temperature, the alkali chlorides may be volatile and may be lost by this process. The total ash usually consists of carbonates, phosphates, silicates and silica which include both physiological ash – which is derived from the plant tissue itself and non-physiological ash-which is the residue of the adhering material to the plant, e.g., sand and soil.
Indian Pharmacopoeia (IP) 2006, prescribes suitable methods for the determination of ash values.

Method
Weighed accurately 2-3 g of the air dried crude drug in the tarred platinum or silica dish and incinerated at 350^{o}C until free from carbon, cooled and weighed. Calculate the percentage of ash with reference to the air dried drug.

Acid insoluble ash
Ash insoluble in hydrochloric acid is the residue obtained after extracting the sulfated of total ash with HCL, calculated with reference to 100 g of drug. For the determination of acid insoluble ash as prescribed in IP 1996, method I is used unless otherwise directed in the individual.

Method
Boiled ash with 25 ml of 2M HCL for 5 minutes, collected the insoluble matter in an ash less filter paper, washed with hot water, ignited, cooled in a desiccator and weighed. Calculate the percentage of acid insoluble ash with reference to the air dried drug.

Water soluble ash
Water soluble ash is that part of the total ash content which is soluble in water. It is good indicator of either previous extraction of the water soluble salts in the drug or incorrect preparations. Thus, it is the difference is weight between the total ash and the residue obtained after treatment of Total ash with water.

Method
Collected the insoluble matter in an ash less filter paper, washed with hot water and ignited for 15 minutes at temperature 350^{o} C. Weight of the insoluble matter subtracted from the weight of the ash the ash. The difference of weight represented the water soluble ash. Calculate the percentage of water soluble ash with reference to the air dried drug.

Determination of extractive values (Raina MK, 1998, Sarine YK, 1993, Standardization of single drugs of Unani Medicine, 1983)
This method determines the amount of active constituents in a given quantity of medicinal plant material when extracted with solvents. It is employed for that material for which no chemical or biological assay method exist.[14]

Drying and size reduction
The dried Root of Aloe-vera subjected to 1 weak. The dried Root was further crushed into powder form was ready for extraction process.

Preparation of aqueous extract
The Root of BARBADENSIS MILLER ROOT Lindl. Ex. Wall air dried for 1 week and then grinds into powder followed by soaking (1:20, w/v) in distilled water for 72 h. The aqueous extract of Aloe vera BARBADENSIS MILLER ROOT Lendl. Ex wall obtained was filtered using Whatman filter paper and regarded as the stock solution (with 100% concentration). The stock solution was then diluted using dH2O to concentrations of 10 and 50% for the pharmacological studies. [11] A volume of 100 ml of crude dried (percentage of yield is= 30). Based on the amount of crude dried obtained, it was estimated that the 10, 50 and 100% mg/kg, respectively.[19]

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Preparation of red blood cells (RBCs) suspension

5 ml blood was collected in EDTA vials from healthy human volunteer who has not taken any NSAIDS for 2 weeks prior to the experiment then centrifuged at 3000 rpm for 10 min and separated packed cells were washed three times with equal volume of normal slain. The volume of blood was measured and reconstituted as 10% v/v suspension with normal slain.(7)

Human red blood cells (HRBC) membrane stabilization method – The principle concerned in the method is stabilization of human red blood cell membrane by hypo tonicity induced membrane Lysis. Blood was collected (2 ml) from healthy volunteers and was mixed equal volume of sterilized alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% NACL in distilled water) and centrifuge at 3000 rpm. The packed cells were washed with isosaline solution and a 10% suspension was prepared normal saline and kept at 4^0 C different concentration of ALOE VERE (BARBADENSIS MILLER) ROOT lindl. Ex. Wall extract (50 µg/ml, 100 µg/ml) and control distilled water instead of hypo saline to produce 100 % hemolysis were separately mixed with 1 ml of phosphate buffer, 2ml of hypo saline And 0.5ml o 10% HRBC suspension were added to prepare. All the assay mixture were incubated at 37° C for 30 min. and centrifuge at 3000 rpm for 20 min at 25° C and hemoglobin content of the supernatant solution was estimated spectrophto metrically at 560 nm. The percentage of HRBC membrane stabilization or protection was calculated by using the following formula (kar et at.2012.)

$$\% \text{ Inhibition} = 100 - \frac{\text{Optical density of drug}}{\text{Optical density of control}} \times 100$$

Table no 1- In vitro anti-inflammatory activity BY HRBC membrane stabilization method

S.NO.	TYPE OF EXTRACT	CONCENTRATION µg/ml	ABSORPTION (nm)	% INHIBITION OF DENATURATION
1	Control	--	2.630	
2	Aqueous (chloroform)	50 **(µg/ml)**	0.680	74.14%
3	Aqueous (chloroform)	100 **(µg/ml)**	0.530	79.84%
4	Aqueous (ethanol)	50 **(µg/ml)**	0.873	66.80%
5	Aqueous (ethanol)	10 **(µg/ml)**	0.650	75.25%
6	Aqueous (Acetonitrile)	50 **(µg/ml)**	0.680	74.14%
7	Aqueous (Acetonitrile)	100 **(µg/ml)**	0.550	20.8%

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8	Diclofenac	50 **(µg/ml)**	0632	75.96%
9	Diclofenac	100 **(µg/ml)**	0.520	80.22%

Table no. 2: different ash values of ALOE VERA ROOT.		
S. No.	**Types of ash value**	**Observation (%) w/w**
1	Total ash	16.80-17.00%
2	Acid insoluble ash	4.80-5.20%
3	Water soluble ash	2.60-2.80%

Phyto-Chemical Screening:

Phyto-chemical screening also reveals the presence of flavonoids, saponins, tannins, phenols etc. mainly tri-terpenoids which plays the prominent role for their therapeutic potential in the drug as reported in the literature.

Phyto constituents in different extract:

S.N.	Test	Method	Chlorofom extract	Ethanol extract	Water extract
1	Carbohydrates	Molish test	-	+	+
		Fehling test	-	+	-
		Benedict test	+	-	+
2	Alkaloids	Dragondroffs test	+	-	+
		Mayer's test	+	+	-
		Wagener test	+	+	+
		Picric acid test	+	+	-
3	Tannins	Ferric chloride test	-	+	-
		Nitric acid test	+	+	-
		Iodine solution test	+	-	+
4	Flavonoids	Shinoda test	+	+	-
		Test with lead acetate solution	+	+	-
		Test with sodium hydroxide	+	-	+
		Picric acid test	+	+	-
5	Saponins	Foam test	-	+	+
		Hemolytic	+	+	-
6	Glycosides	Killer killiani test	-	-	+
		Brontrager test	-	+	-
		Legal test	-	+	-
7	Mucilage	Ruthenium red	-	+	-

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6	Glycosides	Test with sodium hydroxide	+	-	+
		Picric acid test	+	+	-

Discussion

The HRBC membrane stabilization method was used for the in-vitro anti-inflammatory activity of the different extract of Root of Aloe-vera (Barbadensis miller) Root. All extract showed more activity then the standard drug. Aqueous extract showed maximum activity (i.e & 75% inhibition of denaturation in hypotonic solution) at the concentration of 50 µg/ml) 74.14% as the comparison of the standard diclofenac 50 µg/ml show 75.96 %inhibition of denaturation.

Summary & Conclusions

Non polar & polar extract of Aloe-Vera (*Barbadensis miller*) roots exhibited membrane stabilization effect by inhibiting hypo tonicity induced lysis of erythrocyte membrane. The erythrocyte membrane is analogues to the Lysosomal membrane and its stabilization implies that the extract may as well stabilize lysosomal membrane and its stabilization of lysosomal membrane is important in limiting the inflammatory response by preventing the release of lysosomal constituent of activated neutrophil such as bacterial enzymes and proteases which cause further tissue inflammation and damage. From the above study it was concluded that the water extract of eucalyptus Root has significant membrane stabilization property.

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