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# *TERMINALIA ARJUNA* EXTRACT IMPREGNATED COLLAGEN DERMAL FILMS FOR WOUND HEALING

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**Keywords:**

Collagen, *Terminalia arjuna* bark extract, Micro shrinkage Temperature, Wound Healing Studies, Antioxidant efficiency.

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## ABSTRACT

The purpose of this research work is to formulate a collagen based dermal films containing *Terminalia arjuna* bark extract to improve the quality of wound healing. For this, Collagen was isolated from bovine Achilles tendon and tested for its confirmation of presence, purity and sterility. The physicochemical compatibility between collagen and *Terminalia arjuna* extract was studied by FT-IR and results revealed no interaction. Collagen films were formulated using different concentrations of *Terminalia arjuna* bark extract (1%w/v, 1.5%w/v,2%w/v).The films thus formulated were subjected to various physical, biochemical & wound healing studies. The Micro shrinkage Temperature of the films containing 1%w/v, 1.5%w/v & 2%w/v *Terminalia arjuna* bark extract were found to be $72^0$c,$74^0$C & $79^0$C respectively indicating more hydrothermal stability with the increase in the concentration. Antioxidant oxidant studies revealed the effective utilization of the free radicals by *Terminalia arjuna* incorporated collagen films. Wound Healing studies were performed on Male wistar rats for a period of 7 days and 41.66%,59.36%,55.41%,67.13%,77.02%,76.60% of wound reduction was observed in Control, Marketed (KOLLAGEN$^{TM}$), plain scaffold, 1%w/v, 1.5%w/v, & 2%w/v *Terminalia arjuna bark* extract incorporated collagen film (TAEICFS) treated groups. This research provides a rationale for the topical application of TAEICFS as a feasible and productive approach to improve in the quality of dermal wound healing.

## INTRODUCTION:

Wound healing is a complex process. Repair of skin involves a series of events which are initiated by mechanical, chemical, bacteriological, viral and other traumatic stimuli.

The major steps in the healing of the wound include inflammation, proliferation and migration of different cell types [1]. Inflammation, the first phase occurs immediately after injury and is known as Coagulation which results in a coordinated flux of neutrophils at the wound site. These cells have a natural tendency of respiratory burst mechanism and produce free radicals. Certain non phagocytic cells of the wound generate radicals by the non phagocytic NAD(P)H oxidase mechanism making the wound site rich in oxygen and nitrogen. These free radicals present cause oxidative stress to the system giving pavement to lipid peroxidation, DNA breakage and enzyme inactivation including the free radical scavenging enzymes [2]. There is a substantial evidence of antioxidants playing a vital role in therapy against the pathogenesis of many of diseases caused by oxidants [3] .*Terminalia arjuna,* a naturally occurring root extract rich in tannins,Triterpenoid saponins and flavonoids has shown to posses several biological properties including antioxidant property [4]. This antioxidant property aids in wound healing by free radical scavenging activity. However the delivery of this extract is a matter of concern. *Terminalia arjuna* bark extract incorporated collagen in the form of scaffold ensures slow release of the extract providing the better therapy by acting as a physical support for cellular proliferation. Moreover, collagen itself acts as a wound healing agent possessing biodegradable and biocompatible properties which provide the synergistic activity in significant wound healing along with the extract.

## MATERIALS AND METHODS

***Materials:***

*Terminalia arjuna bark* extract was received as a gift sample from Chemiloids Laboratories Ltd.,

(Vijayawada, India), Glycerin and glacial acetic acid were purchased from S.D. Fine Chemicals Ltd., (Mumbai, India). Other materials used in the study were of pharmaceutical analytical grade. Double-distilled water was used throughout the study.

***Methods:***

***Investigations of physicochemical compatibility of Terminlia arjuna bark extract and collagen:***

Collagen was isolated from Bovine Achilles tendon, using 0.5M acetic acid and 5%w/v Nacl solution following the previously reported procedure[5]. The physicochemical compatibility between *Terminalia arjuna* bark extract and collagen was studied by using Perkin Elmer Fourier Transform Infra Red (FTIR) Spectroscopy. The infrared spectra were recorded using Perkin Elmer Fourier Transform Infra Red (FTIR) Spectrometer, Shelton, USA, by using KBr pellet method and spectra were recorded in the wavelength region between 4000 and 400 $cm^{-1}$. The spectra obtained for *Terminalia arjuna* bark extract, collagen and physical mixture of *Terminalia arjuna bark* extract with collagen were compared.

***Development of Terminalia arjuna bark extract Impregnated Collagen Dermal films):***

*Terminalia arjuna* aqueous bark extract with concentrations 1%w/v, 1.5%w/v, 2%w/v (extract previously dry heat sterilized) was solubulised in 3ml of absolute alcohol. Each of the prepared solutions were mixed with 40ml of collagen viscous solution (concentration of collagen adjusted to 11mg/ml with 0.05m acetic acid) by constant stirring for 24hrs at $4^0$C. The obtained mixture was than squeezed through muslin cloth to remove any precipitate formed during the process. The viscous dispersion thus obtained was deareated by sonication and casted on flat circular glass platform cup ($64cm^2$) with a polyethylene membrane base and placed in an incubator maintained at $37^0$C until dried. The scaffold thus obtained was sterilized under UV- Radiation [6], for a period of 18hrs, stored in a dessicator and used for further experimental purpose

***Microbial Test [7]:***

The presence of microorganisms from the film was tested by the direct inoculation method. For the microbial studies Nutrient agar media was prepared, sterilized and transferred to 5 Petri plates, each containing 25ml of nutrient agar media and numbered 1, 2, 3,4 & 5 respectively. Plate 1 was maintained as control, Plates 2 was inoculated with Plain Scaffold. Plate 3, 4 & 5 were inoculated with 1%w/v, 1.5% w/v & 2% w/v TAEICFS respectively. The 5 Petri plates were incubated at 37$^{o}$c for 24 hours and observed for the presence of microorganisms.

*2.2.4* ***Evaluation of Physicochemical Properties****:*

*2.2.4.1* ***Thickness****:*

The thickness of the TAEICFS was measured by a screw gauge (LINKER-20 X 1/100 mm).

*2.2.4.2* ***Folding Endurance****:*

Folding endurance was measured manually for the prepared films. For this a strip of film (2x2 cm$^2$) was cut evenly and repeatedly folded at the same place until it broke. The number of times the film could be folded at the same place without breakage gave the exact value of Folding Endurance.

***2.2.4.3 Scanning Electron Microscopic (S E M) Studies****:*

The films were subjected to SEM analysis for the determination of the topographical and morphological characteristic features. For this *Terminalia arjuna* bark extract incorporated dried collagen membrane was mounted on a standard SEM sample holder and fixed to the base. The sample was then sputtered coated with platinum coating using a SEM coating system before viewed under JEOL 6380 scanning electron microscope with accelerating voltage of 10.

***2.2.4.4 Water Vapor Transmission Test, [8]:***

For this study Glass vials of equal diameter were used as transmission cells. These cells were washed thoroughly and dried in an oven. About 1 gram of fused calcium chloride was placed in the cells and the film measuring 2.836 cm$^2$ was fixed over the brim with the help of an adhesive. The cells were weighed accurately and initial weight was

recorded and then kept in a closed desiccator containing the saturated solution of potassium chloride (200 ml).Then the cells were taken out and weighed after 6,12,24,48 and 72 hours. From the increase in the weights the amount of the water vapor transmitted and rate at which water vapor transmitted was calculated using the formula

$$Q = WL/S$$

Q = Water vapor transmission coefficient (g/cm/ 24h)

W = Weight of water vapor transmitted (g / 24h)

L = Thickness of the patch (mm)

S = Exposed surface area of the patch ($cm^2$)

***2.2.4.5 Micro Shrinkage Temperature Studies, [9]:***

The Micro shrinkage Temperature measurements were carried out for the plain film and TAEICFS. For this, the collagen films were stage fitted to an optical microscope. A small piece of collagen film was moistened with a drop of water on a glass slide and heated constantly with the help of a tungsten lamp. The temperature at which the film started to shrink was viewed through the microscope and was noted as Micro shrinkage Temperature.

***2.2.4.6 Equilibrium Swelling Ratio Determination, [10]:***

The equilibrium swelling ratio ($E_s$) was measured by the conventional gravimetric method. The dry weight of film was measured before immersing in 0.05 M phosphate buffer saline (PBS) pH 7.4 at a temperature of $37^0$ C and excess surface phosphate buffer saline was blotted out with absorbent paper. The wet weight ($W_s$ ) of the film was determined after being incubated for 24 hours. The equilibrium swelling ratio of the films was defined as the ratio of weight increase ($W_s - W_d$ ) with respect to the initial weight ($W_d$ ) of dry samples. Each value was averaged from three parallel measurements. $E_s$ was calculated using the following equations:

$$E_s = \frac{Ws - Wd}{Wd}$$

Where $W_s$ and $W_d$ denote the weights of swollen and dry samples, respectively.

*2.2.4.7* ***Antioxidant Efficiency [7]:***

Cellulose paper was dipped in a solution containing Oleic acid in hexane (0.1 M). After adding the initiator AIBN, the oxidation of Oleic acid was monitored by measuring the absorbance at 234nm for 30 min. The TAEICFS were placed over the cellulose paper separately containing Oleic acid and the experiment was repeated. For the control experiment the oleic acid solution (0.1 M) treated with AIBN initiator was used.

2.2.4.8 ***Skin irritancy test using rabbit***:

A primary skin irritation test was performed since skin is the vital organ to which the extract is reacted. The test was carried on a healthy rabbit weighing between 1.5 to 2kg. The test was conducted on unabraided skin of rabbit. The control film (collagen alone) was placed on the left dorsal surface of the rabbit; whereas the test (TAEICFS) was placed on the right dorsal surface of the rabbit. Before placing the films the unbraided skin was cleaned with rectified spirit. The films were removed after 24hrs and the skin was examined for erythema /oedema

*2.2.4.9* ***Wound Healing Studies on Male Wistar Rats [8]:***

Male Wistar rats weighing 180-200g obtained from the animal house of the Bapatla College of Pharmacy (1032/ac/07/CPCSEA) Bapatla, were maintained at constant temperature of 26 ± $2^{o}$ C and humidity at 30-40% with 12hrs light and dark cycle through out the experiment. The animals were housed in clean polypropylene cages in an air-conditioned animal house were fed with commercial rat feed and sterile water. The experiment protocol IAEC/II/15/BCOP/2009 was approved by Institutional Animal Ethical Committee (IAEC) of Bapatla College of Pharmacy. Animals were divided into six groups, each group comprising of six rats and the following groups were made.

Group 1: For control.

Group2: For Marketed Formulation (KOLLAGEN$^{TM}$).

Group 3: For Plain collagen films

Group 4: For 1% w/v *Terminalia arjuna bark extract* impregnated collagen films.

Group 5: For 1.5% w/v *Terminalia arjuna bark extract* impregnated collagen films.

Group 6: For 2% w/v *Terminalia arjuna bark extract* impregnated collagen films.

The area was cleared off hair by using a depletory and anaesthetized using chloroform. A metal template measuring 1x1 cm (0.785$cm^2$ area) was placed on the stretched skin and an outline of the template was traced on the skin using a fine tipped pen. The wound was made by excision wound technique. The plain film, KOLLAGEN$^{TM}$ standard (Sheet) and TAEICFS of different concentrations were applied separately on the excised wounds to the animals of groups.

## 3. Results and Discussion

*3.1. Physicochemical compatibity of Teminalia arjuna* bark extract *and collagen:*

The IR spectra of *Terminalia arjuna* bark extract alone showed the principal peaks at wave numbers 1699$cm^{-1}$,1446 $cm^{-1}$, 1361 $cm^{-1}$confirming the purity of the *Terminalia arjuna bark* extract. In the IR spectra of physical mixture of *Terminalia arjuna bark* extract and collagen the major peaks of *Terminalia arjuna bark* extract were observed at wave numbers 1660 $cm^{-1}$, 1445 $cm^{-1}$, 1361$cm^{-1}$.. However, some additional peaks were observed with the physical mixture, possibly because of the presence of collagen. These results suggested that the *Terminalia arjuna bark* extract and collagen were compatible.

### *3.2. Microbial studies:*

The microbial tests conducted on various collagen films by direct inoculation method showed no growth of microorganisms in nutrient agar medium indicating the extract lodged film was sterile and safe to use.

### *3.3. Physicochemical characterization of films:*

The results of the physicochemical characterization of the films are tabulated . The thickness of the films was found to be slightly increased with the increase in concentration. Folding endurance study indicated that the films could withstand rupture. Swelling index study results indicated that the films had a significant impact on the absorption of

wound exudates. The increased hydrophilic concentration of the extract in the film increased the water vapor transmission rate. The higher shrinkage temperature of TAEICFS suggests increased hydrothermal stability when compared to plain collagen films.

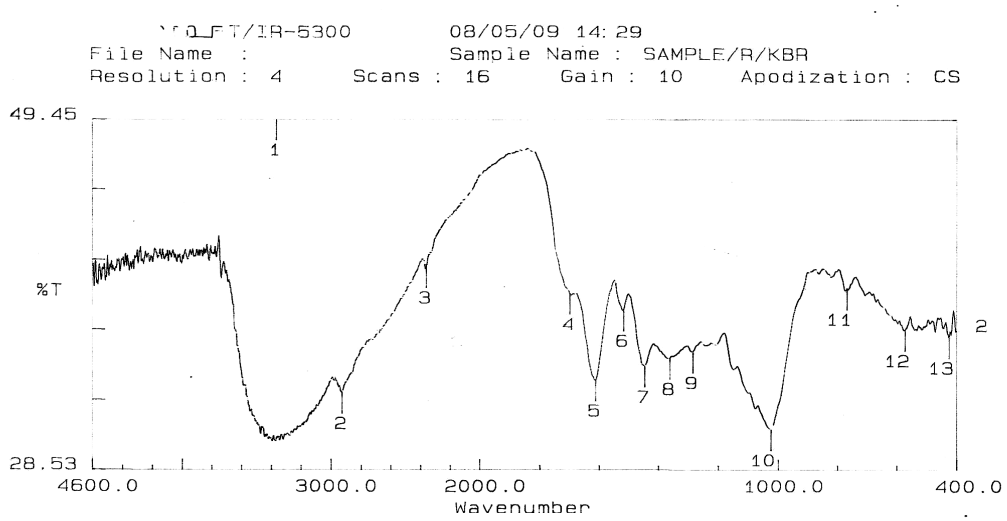
### *3.4. Test of antioxidant efficiency:*

Time dependence absorbance study clearly indicated the scavenging action of *Terminalia arjuna bark* extract against peroxy radicals. Oleic acid impregnated cellulose paper on treatment with radical initiator, 2,2' Azo bis iso butyro nitrile(AIBN), resulted in conjugated diene (oxidized form) that absorbs at 234 nm. When TAEICFS were placed over the impregnated cellulose paper, the extract monetarily stopped the peroxidation of oleic acid. The immediate decrease in absorbance after addition of the incorporated extract might be due to the reaction of *Terminalia arjuna bark* extract with free radicals, preserving the unsaturated fatty acid chain from further per oxidation.

### *3.5. **Wound Healing Studies on Male Wistar Rats:***

The wound healing studies when conducted in Male Wistar Rats, found a markable decrease in the wound area when treated with TAEICFS and Marketed Formulation (KOLLAGEN$^{TM}$) when compared to that of the control group. Further, the wound area significantly reduced in the groups treated with TAEICFS with all concentrations(1.5%w/v TAEICFS found to be most effective) than the group treated with marketed dosage form (KOLLAGEN$^{TM}$).This might be due to the free radical scavenging property of *Terminalia arjuna bark extract* acting as antioxidant, which aids in wound healing and protect tissues from oxidative damage. Moreover, the collagen in the films enhances the cellular proliferation in wound healing. Thus, the *Terminalia arjuna bark extract* and collagen in the form of film act synergistically resulting in improved and quicker wound healing.

## IR OF *Terminalia arjuna bark extract*

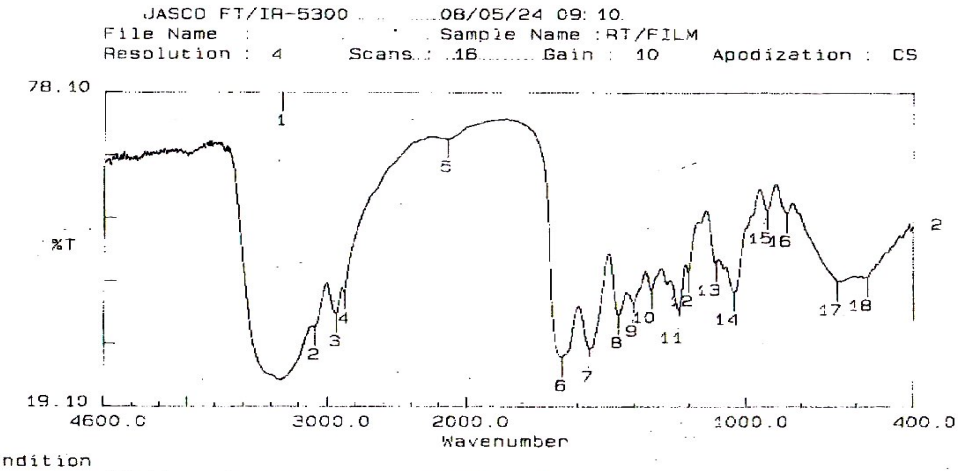



Condition
upper 49.45 lower 28.53 depth 1.00

Peak table

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1: | 3369.94 ( 30.3) | 2: | 2926.28 ( 33.2) | 3: | 2361.08 ( 40.7) | 4: | 1699.44 ( 39.1) |
| 5: | 1612.64 ( 34.0) | 6: | 1518.11 ( 38.1) | 7: | 1446.74 ( 34.7) | 8: | 1361.87 ( 35.2) |
| 9: | 1284.71 ( 35.6) | 10: | 1024.29 ( 31.0) | 11: | 767.74 ( 39.4) | 12: | 572.91 ( 36.9) |
| 13: | 426.31 ( 36.5) | | | | | | |

## IR OF COLLAGEN AND *Terminalia arjuna bark extract*




Condition
upper 78.10 lower 19.09 depth 1.00

Peak table

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 1: | 3439.51 ( 24.0) | 2: | 3369.45 ( 34.1) | 3: | 2937.85 ( 36.6) | 4: | 2876.12 ( 41.0) |
| 5: | 2145.04 ( 69.4) | 6: | 1660.07 ( 28.3) | 7: | 1554.55 ( 30.0) | 8: | 1445.32 ( 36.4) |
| 9: | 1404.30 ( 38.6) | 10: | 1361.72 ( 41.2) | 11: | 1240.34 ( 37.3) | 12: | 1205.62 ( 44.3) |
| 13: | 1107.24 ( 46.4) | 14: | 1043.58 ( 40.8) | 15: | 923.99 ( 56.0) | 16: | 852.84 ( 55.5) |
| 17: | 669.36 ( 42.6) | 18: | 561.34 ( 43.6) | | | | |

**Table I: Physicochemical Properties of TAEICFS***

| Type of Formulation | Thickness (µm) | Folding Endurance | W.V.T. Coefficient (Q,g/cm/day) | M.S.T. ($^0$C) | S.I [(mg/mg)/ 24 hrs] | A.O.E (%) |
|---|---|---|---|---|---|---|
| **Plain scaffold** | 36.33±0.45 | 412.66±0.1 | $3.6\times10^{-4}$ | 64±0.11 | 4.850±0.04 | 90.58 |
| **1%w/v TAEICFS** | 34.66±0.47 | 392.33±0.3 | $3.05\times10^{-4}$ | 72±0.24 | 4.621±0.06 | 92.84 |
| **1.5%w/vTAEICFS** | 36.00±0.78 | 387.33±0.9 | $4.12\times10^{-4}$ | 74±0.06 | 5.026±0.04 | 95.06 |
| **2%w/v TAEICFS** | 40.00±0.74 | 384.66±0.3 | $5.09\times10^{-4}$ | 79±0.12 | 5.201±0.07 | 95.01 |

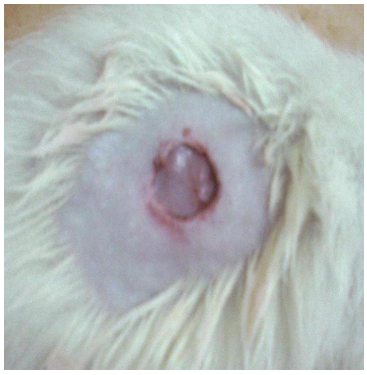
*All Values are expressed as mean ± SD (n=10). TAEICFS indicates *Terminalia arjuna* bark extract incorporated collagen dermal films; W.V.T, Water Vapour Transmission; M.S.T, Microshrinkage Temperature; S.I, Swelling Index; A.O.E, Antioxidant Efficiency;

**Table II: Observed wound reduction**

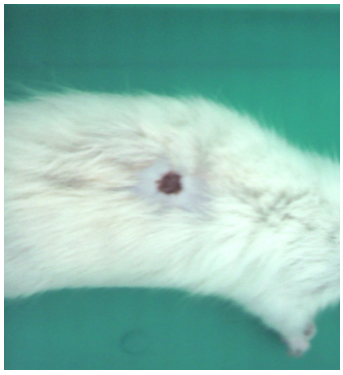
| Group | | I Control | II $F_1$ | III $F_2$ | IV $F_3$ | V $F_4$ | VI $F_5$ |
|---|---|---|---|---|---|---|---|
| Wound area ( $cm^2$ ) | Day 0 | 0.785 | 0.785 | 0.785 | 0.785 | 0.785 | 0.785 |
| | Day 7 | 0.458±0.05 | 0.319±0.04 | 0.322±0.02 | 0.258±0.03 | 0.179±0.04 | 0.185±0.02 |
| % wound reduction | | 41.66 | 59.36 | 58.41 | 67.13 | 77.02 | 76.60 |

All Values are expressed as mean ± SD (n=10). $F_1$ indicates Marketed (KOLLAGEN TM) Formulation treated group; $F_2$, Plain Collagen film treated group; $F_1$, 1%w/v TAEICFS treated group; $F_4$, 1.5%w/v TAEICFS treated group; $F_5$, 2% w/v TAEICF treated group.

**Wound Healing Studies:**

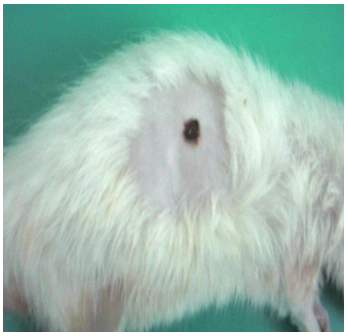


**i) Control Group**

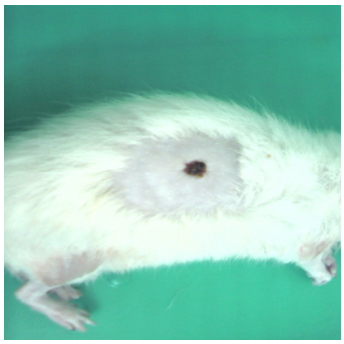


ii) **Marketed Brand (KOLLAGEN ™)**

**Treated Group**



**ii )$F_2$ - Plain Collagen Film Treated Group**



**iv) $F_4$ 1.5%w/v TAEICF Treated Group**

## 4. Conclusion

The developed *Terminalia arjuna bark extract* incorporated Collagen based films enhance the wound healing process.


## Acknowledgement

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## References


[1]. Hunt, Thomask, Hopf, Harriet, Hussain, Zamirul, "Advances in skin & amp; Wound Care", Vol.13, May/June 2000, P: 6-11.

[2]. Wiseman H, Halliwell B. "Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer ", Biochem J, Vol. 313, January 1, 1996, P: 17-29.

[3]. Stephen D Skaper, Michele Fabris, Vanni Ferrari, Maurizio Dalle Carbonare, Alberta Leon, "Querecetin protects cutaneous tissue associated cell types including sensory neurons from oxidative stress induces by glutathione depletion" Cooperative effects of ascorbic acid, Free Radic Biology and Medicine, Vol.22, No.4, 1997, P: 669-678.

[4] Kapoor LD. "Handbook of

Ayurvedic Medicinal Plants". Boca Raton, FL. CRC Press; 1990:319-320.

[5]. Deyl Z, Adam M. "Preparation of insoluble collagen". In: Hall DA, Editor.The methodology of connective tissue research.1st ed.Oxford: Joynson-Bruvvers; 1976, P: 1–7.

[6]. Alina Sionkowska, Joanna Skopin ska Marcin Wisniewski, "Photochemical stability of collagen/poly(vinyl alcohol) blends", Polymer Degradation and Stability Volume 83, Issue 1, January 2004, P: 117–125.

[7].K.VijayaLakshmi, R.Judith and Suseela Rajakumar, "Novel Plant based formulations for short term preservation of animal skins". Journal of Scientific & Industrial Research, Vol-68, August 2009, P: 699-707.

[8].Udhumansha Ubaidulla, Molugu V.S. Reddy, Kumaresan Ruckmani, Farhan J. Ahmad, and Roop K. Khar, "Transdermal Therapeutic System of Carvedilol: Effect of hydrophilic and hydrophobic matrix on *invitro* characteristics", AAPS Pharmasci tech, Jan 19, 2007, Vol-8, Issue-1, P: E1-E8.

[9]. D.Gopinath, M. Rafiuddin Ahmed, K. Gomathi, K. Chitra, P. K. Sehgal and R. Jayakumar "Dermal Wound Healing Processes with Curcumin incorporated collagen films" Biomaterials, Volume-25, Issue 10, May 25, 2004, P: 1911-1917.

[10].Chalonglarp Tangsadthakun, Sorada Kanokpanont, Neeracha Sanchavanakit, Tanom Banaprasert, Siriporn Damrongsakkul, Properties of Collagen/Chitosan Scaffolds for Skin Tissue Engineering, Journal of Metals, Materials and Minerals, Vol-16, No-1, 2006, P: 37-44