ISSN: 2348-8948
Vol: 2; Issue: 5

RESEARCH ARTICLE

WOUND HEALING ACTIVITY OF THE LEAVES OF Trichosanthes cucumerina L. (Cucurbitaceae) ON ex-vivo PORCINE SKIN WOUND HEALING MODEL

Periyanayagam K*, Jegadeesh S, Karthikeyan V, Jancy Gracelet R

Asst Reader, Department of Pharmacognosy, College of pharmacy, Madurai Medical College, Madurai 625 020, Tamil Nadu, India

Date Received:	Date of Accepted:	Date Published:
20th Apr2014	27th Apr2014	12th May2014

Abstract:

OBJECTIVE: To pre-screen the ex- vivo wound healing activity of flavonoid rich fraction of ethyl acetate extract of the leaves of Trichosanthes cucumerina Linn. Family Cucurbitaceae using porcine skin wound healing model (PSWHM) along with phytochemical, EDS, HPTLC analysis. The aim of this present study is to provide pharmacological validation to the traditional claim for wound healing activity of Trichosanthes cucumerina leaves

Keywords: Trichosanthes cucumerina, Cucurbitaceae, Epidermal migration, ex-vivo wound healing, Trace element, Energy Dispersive X-ray Spectrometer, HPTLC

Introduction

Wound healing is the process of repair that follows injury to the skin and other soft tissues. Following injury an inflammatory response occurs and the cells below the dermis begins to increase collagen production. Later the epithelial tissue is regenerated [1]. Wound healing management is a complicate and expensive one. So that research on drug which enhances the wound healing process is a thrust area in drug research [2]. Present days, wound healing regimen mainly synthetic chemical moieties (mostly antibacterial) which possess a wide range of side effects. Therefore research needed on herbals with devoid of side effects which associated with synthetic one and wound healing potential of many of traditional medicinal plants remain unexplored [3]. So there is need of the hour to identify the various medicinal plants or their chemical constituents and formulated into convenient form for treatment and management of wounds. Medicinal plants have been reported to be very beneficial in wound care, promote the rate of wound healing with minimal scar [4].

The T.cucumerina (Pudal leaf) leaves used as alexiteric, astringent, diuretic and emetic. In the Konkan, the leaf juice is rubbed over the liver in liver congestion or over the whole body in remittent fevers, cardiotonic, anti-pyretic, anti-periodic useful for intestinal worms, skin disease, biliousness, emetic, externally applied over bald patches of alopecia[5,6]. The present study investigate the wound healing effect of the TCEAE as it contains flavonoid fraction using ex-vivo porcine skin wound healing model (PSWHM). The phenolics and its derivatives were reported to be effective against viruses, bacteria and fungi, and many diseases including wound healing. The above points prompted us to investigate ethyl acetate extract from the leaves of T.cucumerina a widely available and menacingly wasted part of it. Its medicinal applications are still to be explored well. More over trace elements like Magnesium(Mg), Potassium(K), Calcium(Ca), Phosphorous(P) supports wound healing activity as essential trace mineral are required for cellular growth and replication.

Survey of available literature showed that there was no report available on the trace element content of the leaves. So we have decided to estimate the trace element content of the leaves. Pig skin architecture (in both physiological and anatomical) is similar to human skin [7]. Further PSWHM is an excellent model system due to its high reproducibility, easy to handle, economical, without the need of ethical clearance [8]. Epidermal regeneration is an important part of cutaneous wound healing, causing permanent closure of wound and restoration of essential functions of the skin. This process involving the keratinocyte migration and proliferation at the margin of wound and variety of interaction with component of the dermis. Here we want to emphasise the traditional use of the whole plant, aerial parts for the treatment of diabetes and the several supportive scientific research of this claim as anti-diabetic [9,10]. The common complication of diabetic patient is wound as an adverse effect which is an enormous burden on the health care system, both in terms of cost and intensity of care required. Hence this prompted us to investigate the effect of the flavonoid rich on PSWHM – a novel ex-vivo wound healing model. In this model epidermal or keratinocyte migration was measured.

MATERIALS&METHODS:

Pig ears (6month old), Biopsy punch (6mm, 3mm); Phosphate Buffer Solution (PBS), 70% ethanol, Hemotoxylin/eosin, Ethyl acetate extract of *T.cucumerina* leaves (TCEAE), Mupirocin ointment. All chemicals used are SD fine chemicals. For the determination of trace element by Energy Dispersive X-ray Sectrophotometer and CAMAG HPTLC with win CATS 1.4.3 software, densitometry TLC scanner (520nm) was used for HPTLC analysis CO2 incubator.

COLLECTION AND AUTHENTICATION OF THE LEAVES OF *T.cucumerina*:

The leaves of the healthy *T.cucumerina* selected for our study was collected from Tenkasi, Tirunelveli(Dt), Tamilnadu. It was identified, and authenticated by Dr.Stephen, Taxonomist, Dept. of Botany, The American College, Madurai and Dr. Sasikala, Director of Siddha Research Centre, Arunbakkam, Chennai, Tamilnadu, India and. A voucher specimen was deposited at the herbarium of Dept. of Pharmacognosy, Madurai Medical College, Madurai, Tamilnadu, India (PCG-279)
.

PREPARATION OF EXTRACT:

The leaves were dried at room temperature under shade and powdered, sieved (60mesh) and stored in a well closed container. Extracted with ethyl acetate and filtered, evaporated under vacuum (Rotavapor RII, Buchi). The pale green residue obtained (TCEAE) was stored in the refrigerator until further use.

PREPARATION OF TCEAE OINTMENT:

1%, 2%,3% TCEAE ointment was prepared by using simple ointment base IP.

PRELIMINARY PHYTOCHEMICAL SCREENING

Preliminary phytochemical screening was carried out to find out the presence of various phytoconstituents using standard procedure[11-14].

DETERMINATION OF TOTAL PHENOLIC CONTENT :

The total phenolic content of extracts was determined by Folin-Ciocalteau method [15,16]. The extracts were oxidized with Folin-Ciocalteau reagent, and the reaction was neutralized with sodium carbonate. The absorbance of the resulting solution was measured at 760 nm after 20min. Using gallic acid as standard total phenolic content (standard curve was prepared using concentrations 2,4,6,8,10 µg/ml) was expressed as mg GA equivalent/ gram of extract.

ELEMENTAL ANALYSIS BY EDS:

Scanning Electron Microscope model JSM-5610 LV with an EDS microanalysis attachment was used to study the microscopical characters and elements present [17].

PREPARATION OF SOLID SAMPLE:

Mix equal volume of powder and binder pressed up to 30 ton made into pellet. The binder must be free from contaminant element and low absorption. It must stable under vacuum and irradiation conditions.

HPTLC PROFILE OF TCEAE: DEVELOPMENT OF HPTLC FINGERPRINT INSTRUMENT

CAMAG TLC Scanner 3 "Scanner3-070408"S/N 070408(1.41.21) was used for detection and CAMAG Linomat 5 sample applicator was used for the application of the track. Twin trough plate development chamber was used for development of chromatogram. Software was used Win CATS 1.43 at 254nm.

SAMPLE

The TCEAE was dissolved in ethyl acetate to get a concentration of 4, 8 µl of this solution was used for taking HPTLC fingerprint.

STATIONARY PHASE

Aluminium sheets pre-coated with silica gel 60 GF 254 HPTLC plates were used as a stationary phase.

MOBILE PHASE

Toluene: Ethyl acetate:Formic acid: Methanol (3:6:1.6:0.4) was used as the mobile phase for development of chromatogram. The mobile phase was taken in a CAMAG twin trough glass chamber.

DETECTION WAVE LENGTH

The developed plates were examined at wavelength 254 nm in CAMAG TLC scanner 3. The TLC visualization, 3D display of the finger print profile and peak display at 254 nm.

EFFECT OF TCEAE LEAVES ON *EX-VIVO* PORCINE SKIN WOUND HEALING MODEL

Wound healing evaluated by *ex-vivo* porcine skin wound healing model (PSWHM). Porcine (6 months old) ears were obtained from the local slaughter house were washed with PBS and disinfected with 70% ethanol. Circular porcine skin (6mm diameter) taken out from the inner side of the ear by using sterile circular biopsy punch. Subsequently on the excised portion small circular wound (3mm diameter) was made by using sterile circular biopsy punch. Epidermis and upper dermis was removed from the centre for making the wound under sterile conditions. The PSWHM were divided into five groups (n=6). 1%, 2%, 3% test drug (TCEAE) ointment, standard drug (Mupirocin) 2% ointment treated and immersed in PBS along with control in triplicate. The PSWHM kept in CO2 incubator at 37°C for 2 days. Histopathological evaluation was done after staining with hematoxylin / Eosin. The migration was normalized with the PBS group and expressed as mean % ± SE. statistical analysis was performed using one way analysis of variance (ANOVA). p value 0.01 was considered to be statistically significant [18].

RESULTS:

Preliminary phytochemical screening showed the presence of flavonoids, terpenoids, saponins, sterols, alkaloids, proteins and free amino acids. The total phenolic content of TCEAE in terms of gallic acid was found to be 376.5g/g. The leaves were also subjected to elemental analysis by Energy Dispersive X-ray Spectrometer (EDS or EDAX) which was in connection to the SEM. The elemental analysis by EDS showed that the leaves contains inorganic elements like Mg(0.51%), Si(0.35%),P(1.04%),K(5.91%),Ca(5.84%).Determination by HPTLC analysis of TCEAE showed 0.7% apigenin **(Fig 1&2, Table 1)**.

Histopathological evaluation showed all treated wounds were sound with no signs of apoptosis, necrosis or bacterial contamination and no toxicity of the tested concentrations of TCEAE of the leaves. Morphology of the wound margins, epidermis and dermis layer were found to be normal. Epidermal migration or keratinocyte migration distances from the edges of each wound were measured, normalized with the PBS control group and expressed as mean%. The result clearly showed TCEAE (3%) statistically significant wound healing effect which is comparable to the standard drug Mupirocin **(Figure 3&4)**.

DISCUSSION:

Wound healing is a dynamic and complex process in which the tissue layer of damaged tissue & cellular structure are restored into its normal state as closely as possible [32]. Basically healing is the natural body process of regenerating dermal and epidermal tissue [33]. So rapid healing of wound needed to provide suitable conditions that can regenerate the damaged tissue [34]. In recent years, phytochemical constituents of plants with varied pharmacological, physiological and biochemical activities have received attention. Studies have shown that *T.cucumerina* contains many classes of compounds such as flavonoids, terpenoids, saponins, sterols, alkaloids, proteins and free amino acids. The pharmacological studies of *T.cucumerina* leaves showed moderate larvicidal effect in acetone extract[22], anti-bacterial activity against gram positive and gram negative bacteria[23], hair growth promoting activity in rats[24], no toxicity in rats[25]. Plant phenolics act as primary anti-oxidants or free radical scavangers [26]. Lipid peroxidation is an important process in burns, wounds and skin ulcers. Collagen fibrils viability increases by inhibiting lipid peroxidation which cause increases the strength of collagen fibres. Finally prevents cell damage and promotes DNA synthesis [27,28]. Therapeutically potential phenolic compounds possesses anti-infective, anti-inflammatory as well as wound healing by decreasing lipid peroxidation which improve vascularity, increase collagen synthesis and promotes cross linking of collagen [29]. In our study total phenolic and apigenin content of TCEAE was found to be 22.2µg/g GAE 0.7% respectively. EDS study showed the presence Mg(0.51%), Si(0.35%), P(1.04%), K(5.91%), Ca(5.84%). Flavanoids (apigenin) known to have astringent property which is responsible for wound contraction and increased rate of epithelialisation along with the supportive anti-microbial activity . Trace elements are considered the “inorganic switches” in

various medicinal systems. This concept has gained ground in Ayurveda and the traditional medicine. From the reports it is assumed that the higher trace elements content reported in EDS analysis might have also enhance the wound healing property. It is assumed that this effect may be due to the phenolic content, apigenin and the influence of Mg, Si, P, K, Ca content and antioxidant activity. The present finding provide scientific evidence to ethnomedical properties of T.cucumerina leaves in wound healing property. Here we want to emphasise the traditional use of the whole plant and aerial parts for the treatment of diabetes and the several supportive scientific research of this claim as antidiabetic [9,10]. The common complication of diabetic patient is wound as an adverse effect which is an enormous burden on the health care system, both in terms of cost and intensity of care required.

CONCLUSION:

Our study showed significant enhancement of wound repair and therefore can be beneficially, safely used as auxiliary therapy in diabetic patient with foot ulcers in addition to the other available treatment. Further investigations are needed with purified constituents to understand the complete mechanism of wound healing process.

Conflict of interest statement:

We do not have any conflict of interest.

ACKNOWLEDGEMENT

Dr. D. Saravanan, Asst. Prof ,The Department of Chemistry, S.Kavitha, Technician ,Common Instrumentation Facilities National College, Trichy, Tamil Nadu, India for SEM and EDS are gratefully acknowledged.

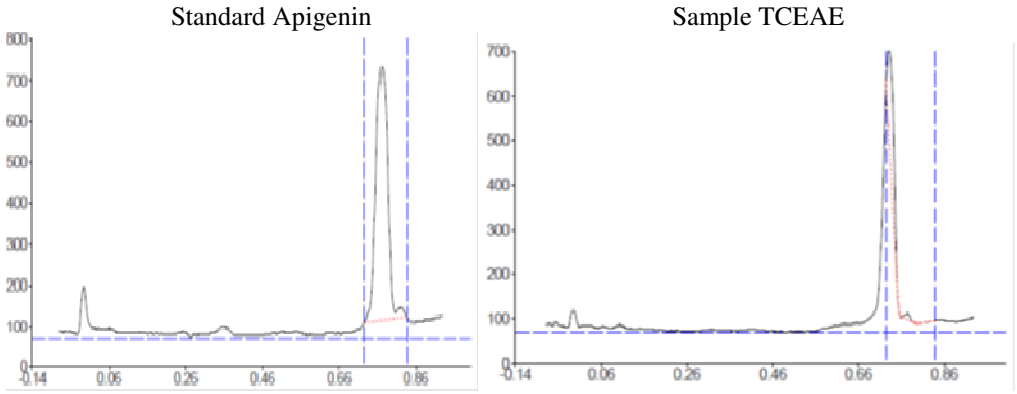

Fig-1 HPTLC peak display

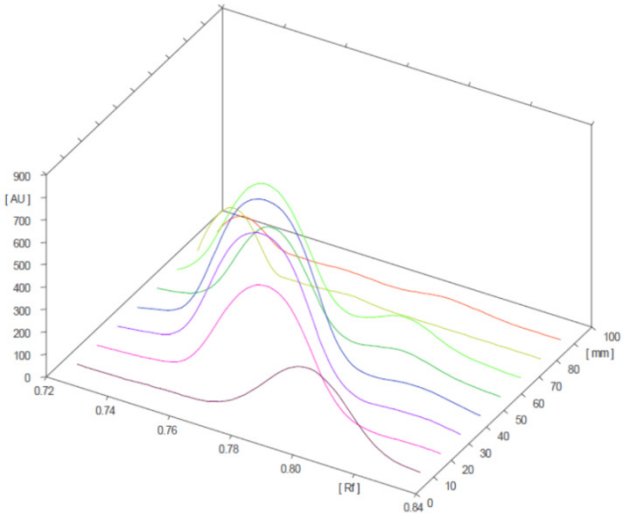

Fig-2: CO-HPTLC profile of TCEAE showing the presence of Apigenin3D display

Table-1Rf value of TCEAE

SI NO	SAMPLES	Start Rf	Max Rf	Max % M	End Rf	Area	Area %
1	Standard Apigenin	0.73	0.77	96.34	0.81	14882.2	97.62
2	Sample TCEAE	0.73	0.76	86.48	0.79	8461.9	88.02

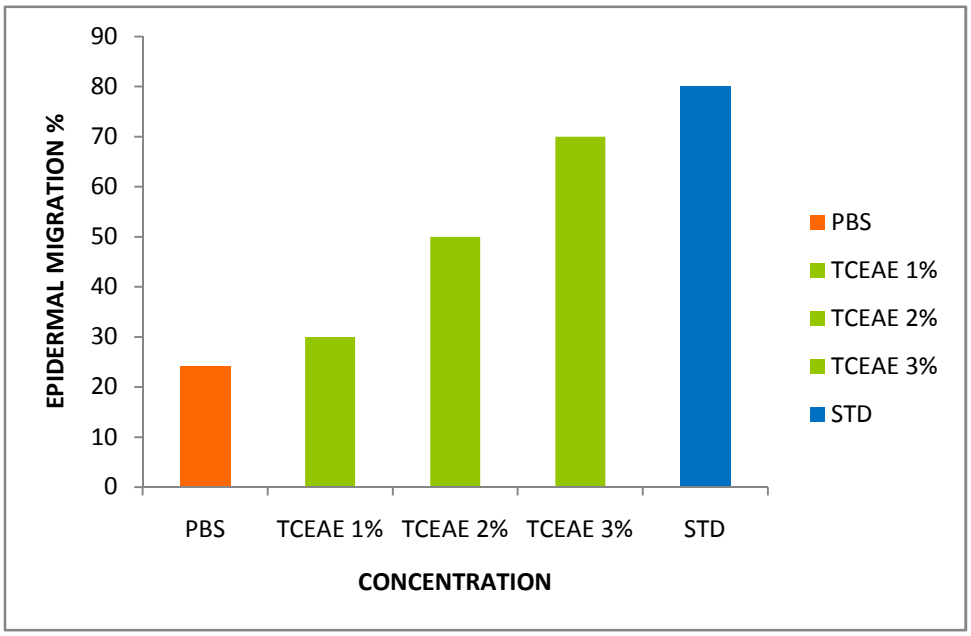

Figure 3: Effect of the TCEAE leaves on *ex-vivo* porcine skin wound healing model

STANDARD (Mupirocin)

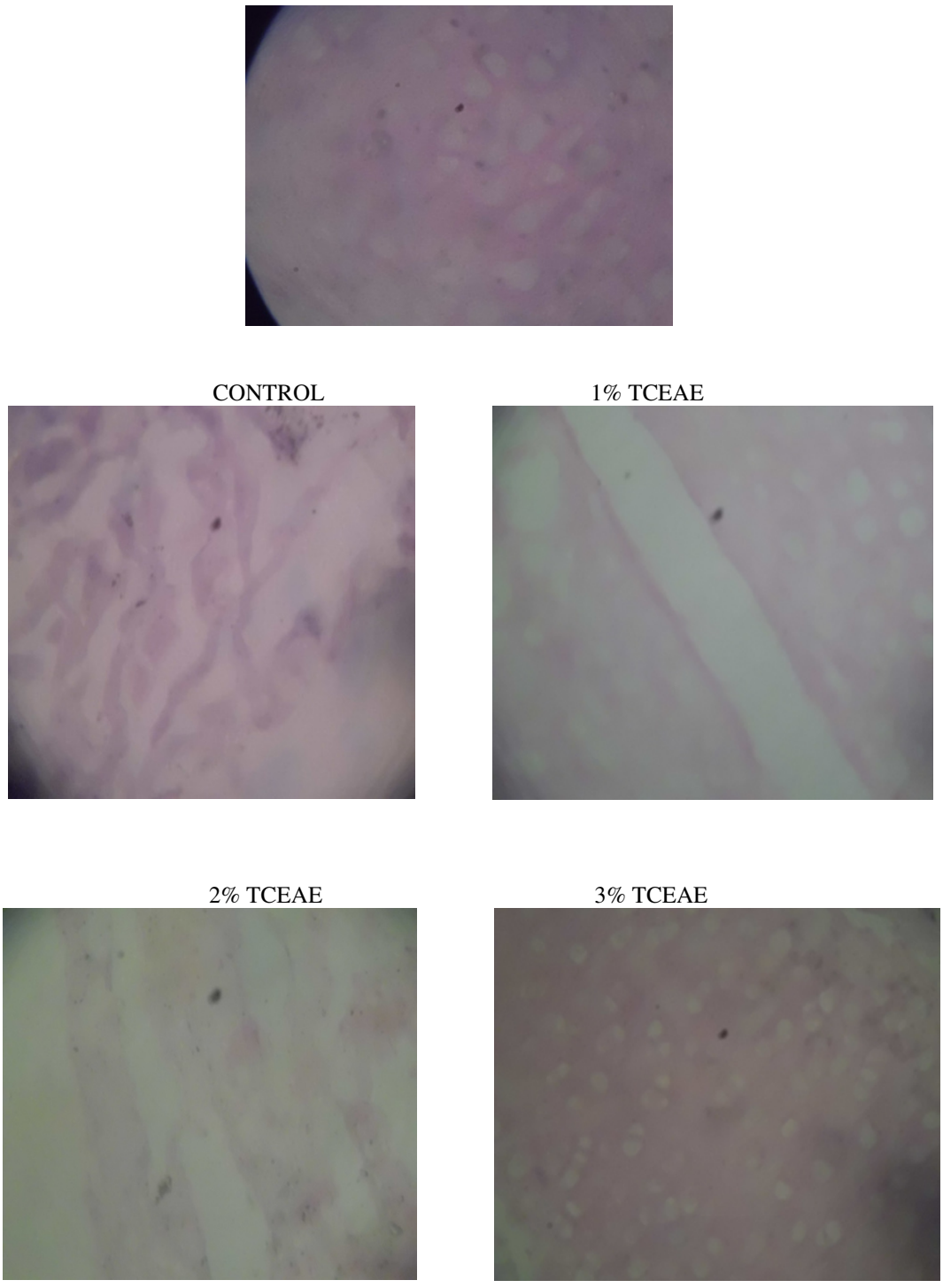

Figure 4: Histology showing epidermal layer migration of PSWHM

REFERENCES:

1. Vijay L, Kumar U. Effect of Moringa oleifera Lam. experimental colitis in rats. Int J Pharm Pharm Sci 2012; 4(1): 337-343.
15. Patil MVK, Kandhare AD, Bhise SD.

on normal and dexamethasone suppressed wound healing. Asian Pac J Trop Biomed 2012; 2(1): S219-23.
2. Ghosh P, Kandhare AD, Gauba D, Raygude KS, Bodhankar SL. Determination of efficacy, adverse drug reactions and cost effectiveness of three triple drug regimens for the treatment of H. pylori infected acid peptic disease patients. Asian Pac J Trop Biomed 2012; 2(2):S783- 89.
3. Gavimath CC, Sudeep HV, Sujan Ganapathy PS, Padmalatha Rai S, Ramachandra YL. Evaluation of wound healing activity of Butea Monosperma Lam. Extracts on rats. Pharmacology online. 2009; 2: 203-216.
4. Kumar B, Vijayakumar M, Govindarajan R, Pushpangadan P. Ethno pharmacological approaches to wound healing- Exploring medicinal plants of India. J Ethnopharmacol. 2007; 114(2):103-113.
5. Sandeep Kumar S, Veeru P ,Arun K ,Ranjit K ,Md Ali. *In-vivo* lethal and cyto toxicity assessment of *T.cucumerina.* Int Res J Pharm.2013;4(4): 185-188.
6. Linn TK .Edible medicinal and non-medicinal plants. Springer Dardrecht Heidelberg, Londan, New York.2012; 2: 401-402.DOI:10.1007/978-94-007-1764-0.
7. WHO.Quality control Methods for Medicinal Plant Materials. 28-35(1998).
8. Chaudhri R.D. Herbal Drug Industry. Eastern publishers. New Delhi. 489-501(1999).
9. Arawwawala LD,Thabrew MI,Arambewala LSR, Ferands N,Guruge LD .Anti-bacterial activity of *T.cucumerina* extracts.Int J pharm & Bio Arc.2011; 2(2) 808-812.
10. Kirana H & Srinivasan BP.*T.cucumerina* linn improves glucose tolerance and tissue glycogen in non-insulin dependent diabetes mellitus induced rats. Indian J Pharmacol.2008; 40(3) 103-106.
11. Heywood VH. Scanning Electron Microscopy, Systemics and evolutionary appplications. Proceedings of an International Symposium held at the Department of Botany. Spl vol.4. University of Reading, Academic Press.London: 1-16(1971).
12. Khamlue R, Naksupan N, Ounaroon A, Saelim N. Skin Wound Healing Promoting Effect of Polysaccharides Extracts from Tremella fuciformis and Auricularia auricula on the ex-vivo Porcine Skin Wound Healing Model. IPCBEE 2012; 43: 93-98.
13. Kumari M, Eesha BR, Amberkar M, babu S, Rajshekar KN. Woundhealing activity of aqueous extract of Crotalaria verrucosa wistar albino rats. Asian Pac J Trop Med 2010; 3(10): 783-87.
14. Patil MVK, Kandhare AD, Bhise SD. Anti-inflammatory effect of Daucus carota root on experimental colitis in rats. Int J Pharm Pharm Sci
27. Gosavi TP, Kandhare AD, Ghosh P, Bodhankar SL. Anticonvulsant activity of Argentum metallicum, a Pharmacological evaluation of ethanolic extract of Daucus carota Linn root formulated cream on wound healing using excision and incision wound model. Asian Pac J Trop Biomed 2012; 2(2): S646-55
16. Pradeep G, Naresh A, Jeyarami Reddy A, Nagarjuna Reddy G, Narayana TV, Ramanarayana Reddy V.2012,'Anti- microbial and anti-diabectic activity of *T.cucumerina* and *prosopischilensis* extract against alloxan induced diabectic rats. Int J Adv Pharm Re**s.**2012;3(6)959-967.
17. Mahesh S Krishna , Jeyakumaran Nair A.Anti-bacterial cytotoxic and anti-oxidant potential of different extracts from leaf, bark, wood of *T.cucumerina*. Int J Pharma Sci Drug Res.2010; 2(2)155-158.
18. Purushotham KG, Arun P, Johnsy Jeyarani J, Vasanthakumari R,Sankar L, Raviprakash Reddy B. Synergistic *in-vitro* anti-bacterial activity of *T.cucumerina* leaves with tetracycline. Int J Pharm Tech Res.2010; 2(1)519-523.
19. Ni Putu A, Driani Astiti and Dewa Ngurah Suprapta.Anti-fungal activity of *T.cucumerina* leaf extract against *Arthrinium phaeospermum*(corda)M.E.Ellis,the cause of wood decay on *Albizia falcataria* (L)fosberg.J Int Soci South Asian Agri Sci.2012;18(1)62-69.
20. Ghareeb MA, Shoeb HA, Madkour MF, Rafahy LA, Mohamed MA, Saad AM.Radical scavenging potential and cytotoxic activity of phenolic compound from *T.cucumerina* Linn', Global J Pharmacol2013; 7(4) 486-497.
21. Nayeem N, Karvekar MD.Analgesic and anti-inflammatory activity of the methanolic extract of the frontal leaves of *T.cucumerina*. Int J Pharmacol.2010; 8(1):1055580/941.
22. Rahuman AA, Venkatesan P.Larvicidal efficacy of five cucurbitaceous plant leaf extracts against mosquito species. Parasitol Res.2008; 103(1): 133-139.
23. Joji RL and Beena J.Evaluation of anti-bacterial activity of mimusops Elengi L flowers and T.cucumerina L fruits. Int J Pharm Pharm Sci.2013;5(3):362-364.
24. Sandhya S ,Chandrasekar J, Vinod KR ,David B. Potentiality of aqueous leaf extract of *T.cucumerina* on hair growth promotion in wistar albino rats. Int J Nat Pdts and Res,2012; 3(1) : 14-19.
25. Sandeep Kumar S, Veeru P ,Arun K ,Ranjit K ,Md Ali. *In-vivo* lethal and cyto toxicity assessment of *T.cucumerina.* Int Res J Pharm.2013;4(4): 185-188.
26. Mittal DK, Joshi D, Shukla S. Antioxidant, antipyretic and choleretic activities of crude extract and active compound of Polygonum bistorta Linn. in albino rats. Int J Pharm Bio Sci 2012; 2(1): 25-31.

homeopathic preparation. Der Pharmacia Lettre 2012; 4(2): 626-37.
28. Santram L, Singhai AK. Preliminary pharmacological evaluation of Martynia annua Linn leaves for wound healing. Asian Pac J Trop Biomed 2011; 1(6): 421- 27.
29. Akkol EK, Koca U, Pesin I, Yilmazer D, Toker G, Yesilada E. Exploring the wound healing activity of Arnebia densiflora (Nordm.) Ledeb by in-vivo models. J Ethanopharmacol. 2009; 124: 137- 41.