

PHYTOCHEMICAL SCREENING AND INVITRO EVALUATION OF WOUND HEALING ACTIVITY, POLYHERBAL PREPARATION USING CHICK EMBRYO MODEL

K. Sudheer Kumar¹, D. Jeslin², Segu Prathyusha³, Uttam Prasad Panigrahy⁴, Sabeena Hussain Syed⁵, JeetendraKumar Gupta⁶

¹Professor, Department of Pharmacognosy, Goenka college of pharmacy, Sikar - Lachhmangarh Road, NH-52, Lachhmangarh (Sikar) - 332315 Rajasthan

²Faculty of Pharmacy, Department of Pharmaceutics, Sree Balaji Medical College and Hospital Campus, Bharath Institute of Higher Education and Research, Chromepet Chennai 600044

³Department of Pharmacognosy, School of Pharmacy, Guru Nanak Institutions Technical Campus (Autonomous), Ibrahimpatnam, Hyderabad, Ranga Reddy (Dt), Telangana 501506

⁴Associate Professor, Department of Pharmaceutical Analysis, Faculty of Pharmaceutical sciences, Assam down town University, Sankar Madhab Path, Gandhi Nagar, Panikhaiti, Guwahati, Assam, India - 781026

⁵Assistant Professor, School of Pharmacy, Vishwakarma University, Kondhwa ,Pune 411038

⁶Associate Professor, Institute of Pharmaceutical Research, GLA University Mathura, Uttar Pradesh, India-281406

Email: sudheerkumarkandibanda@gmail.com

DOI: 10.47750/pnr.2022.13.S08.262

Abstract

Reportedly, plants such as *Cassia angustifolia* and *Tridax procumbens* are used in Siddha medical systems to treat various diseases. Using a chick embryo wound model developed as part of the study, the presented research aimed to determine the wound-healing efficacy of methanol and aqueous extracts of the flowers and leaves. The experimental results show that the methanol extract exhibited good dose-dependent curative efficacy. In the chick embryo chorioallantoic membrane excision wound model, the 300g concentration of polyherbal methanol extract increased wound concentration by 50% compared to the negative control model. Compared to single plants and control groups, the results of the polyherbal Preparation were positive. The extract was also a reliable alternative to animal models for the preliminary screening compounds with wound-healing potential.

Keywords: Angiogenesis, *Cassia angustifolia*, *Tridax procumbens*, Chick embryo, Chorioallantoic membrane, Wound healing.

INTRODUCTION

The main objective of the present study is phytochemical screening and in vitro evaluation of wound healing activity of selected medicinal plants and polyherbal Preparation (*Cassia angustifolia* flower extract, *Tridax Procumbens*). and compare the wound healing potency.

- Select the plants and plant materials.
- To collect plant & plant materials.
- To extract selected plant material with a suitable solvent and suitable extraction methods.
- To screen extracted components for identifying *cassia angustifolia* phytochemicals.
- To screen wound healing properties, selected materials were identified and comparing of (polyherbal Preparation).

□ To record, collect, interpret, collect data, and application of the suitable method to produce evidence for selected medicinal plant materials.

MATERIALS:

Crude extract of cassia angustifolia powder (5gm), Crude extract of Tridax procumbens (5gm), Sterile saline (50ml), Methanol (1000ml), Distilled water (1000ml), Marketed Diclofenac sodium (5µg/ml), Eggs (20).

METHODOLOGY:

Embryo collection: The outer surface of the embryos was cleaned with 75% ethanol and incubated at 37 degrees for the duration of the experiment.

Preparation of saturated filter disk for wound healing: Madhu laboratories was the source of the Whatman No. 1 filter paper. Using a standard 5 mm hole puncher, small discs were created, autoclaved, and stored for future use. The pre-sterilized filter disc was tested with various concentrations of the crude extract, ranging from 100 to 1,000. Madhu laboratories were the source of the Whatman No. 1 filter paper. Using a standard 5 mm hole puncher, small discs were created, autoclaved, and stored for future use. The filter disc was tested with various concentrations of the crude extract, ranging from 100 to 300 g/ml, and the control solutions. As positive and negative controls, 50 g/ml of diclofenac sodium in 4% methanol and sterile saline were used.

Wound assay:

Before use, 75% ethanol was used to sterilize all dissection tools utilized in the assembly. The embryos were incubated for eleven days so the chorioallantoic membrane could mature adequately. The outer shell was wiped with 75% ethanol on the 12th day of incubation to sterilize the surface. Under aseptic conditions, a needle was used to make a tiny hole in the eggshell, and a small window was then cracked open to expose the opaque inner membrane. About 0.5-1 ml of sterile saline was used to make the inner shell membrane transparent. Then, this layer was peeled to reveal the CAM layer. The CAM layer was pulled gently with sterile forceps, and a three mm-diameter excision wound was created in the CAM layer using a small pair of dissecting scissors. The drug-saturated discs were then placed on the CAM of the labeled embryos with their respective concentrations and controls. Finally, the eggshell window was covered with parafilm, and the eggs were returned to the incubator. Up until the fifth post-wound-healing day of observation, wound closure was measured on alternate days.

$$WC\% = \frac{\text{Initial wound size} \times \text{specific day wound size} \times 100}{\text{Initial wound size}}$$

RESULTS AND DISCUSSION

Polyherbal preparation ratio :

A ratio of 1:1 (0.1:0.1g) was selected for the medicinal plant extract. For the polyherbal preparation, 0.1g of cassia angustifolia flower aqueous extract and tridax procumbens leaves aqueous extract, 0.1g of cassia angustifolia methanol extract, and 0.1g of tridax procumbens methanol extract were taken. Preliminary phytochemical screening

Table: No:1. Flower extract of cassia angustifolia

S.No	Tests	Methanolic extract	Aqueous extract
1	Carbohydrates	+	+
2	Reducing sugars	+	+
3	Saponins	+	+

4	Sennosides	+	+
5	Flavonoids	+	+
6	Alkaloids	+	+
7	Tannins	+	+
8	Amino acids	+	+
9	Steroids	+	+
10	Glycosides	+	+

“+” Indicates Presence “_” Indicates Absence.



Fig: No:1. Phytochemical screening of cassia angustifolia flower extract

Table: No:2. Leaves extract of tridax procumbens.

S.No	Tests	Ethanol	Chloroform
1	Carbohydrates	+	+
2	Flavonoids	-	-
3	Alkaloids	+	+
4	Tannins	-	+
5	Amino acids	+	+
6	Steroids	+	+
7	Glycosides	-	-
8	Proteins	+	+
9	Purines	+	+

“+” Indicates Presence “_” Indicates Absence.



Fig: No:2. Phytochemical screening of tridax procumbens leaves extract

Table: No:3. Measurement of internal diameter(ID mm) and wound concentration percentage (WC%) on day five post-treatment with Positive Control (Marketed Diclofenac sodium 50µg/ml), Negative control: (Saline).

Treatment	ID(mm)	WC%
Positive Control(Diclofenac Sodium 50µg/ml)	0.4 ± 0.04	86.667
Negative Control	3 ± 0.01	0



Fig.No:3. Diclofenac Sodium

(Posite Control)



Fig.No:4. Normal Saline
(Negative Control)

The 50µg/ml Of Diclofenac Sodium results in 86.667% of wound healing activity. Normal saline doesn't show any activity.

Table: No:4. Measurement of internal diameter(ID mm) and wound concentration percentage (WC%) on day five post-treatment with different concentrations of the aqueous flower extracts of cassia angustifolia

Table: No:4.1. Aqueous extract of cassia angustifolia flower

Aqueous extract	ID (mm)	WC%
100µg/ml	2.6 ± 0.04	13.333
200µg/ml	2.4 ± 0.08	20
300µg/ml	2.2 ± 0.16	26.667



Fig.No:5. 100µg/ml



Fig.No:6. 200µg/ml



Fig.No:7. 300µg/ml

The Aqueous extract of Cassia Angustifolia flowers 100µg/ml , 200µg/ml , 300µg/ml shows the result of 13.33%, 20% & 26.667% of wound closure activity.

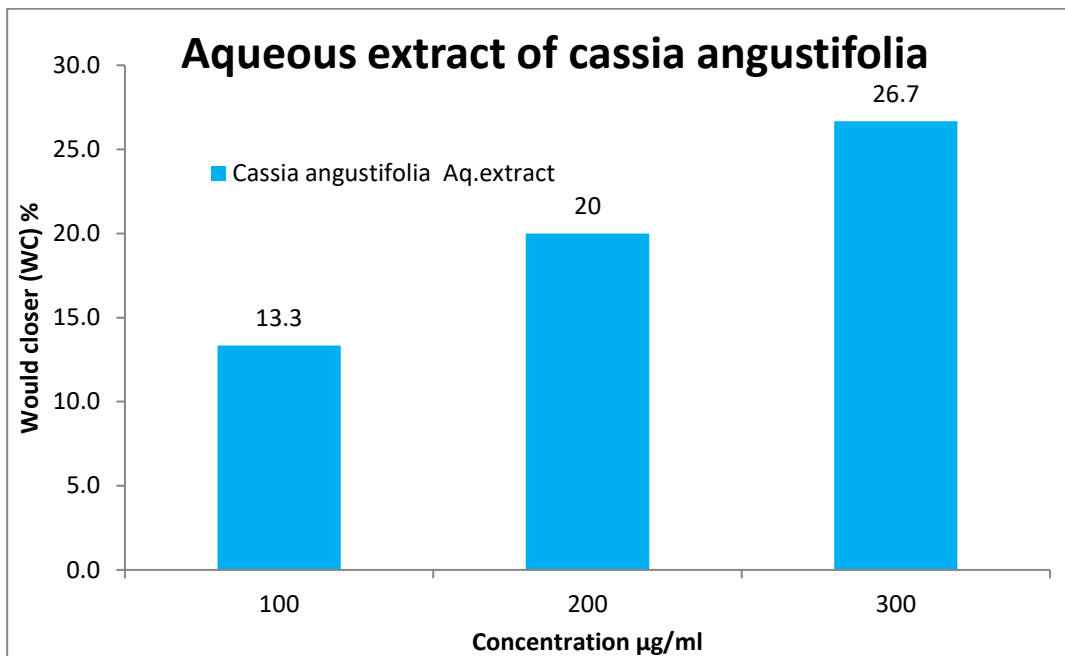


Fig: No:8. Aqueous extract of cassia angustifolia flower

Table: No:4.2. Methanolic extract of cassia angustifolia flower

Methanolic extract	ID	WUC%
100µg/ml	2.5 ± 0.04	16.666
200µg/ml	2.3 ± 0.09	33.333
300µg/ml	1.9 ± 0.04	36.666



Fig.No:9. 100µg/ml

Fig.No:10. 200µg/ml

Fig.No:11. 300µg/ml

The Methanol extract of Cassia Angustifolia flower 100µg/ml, 200µg/ml, and 300µg/ml shows 16.666%, 33.33% &36.66% of wound closure activity.

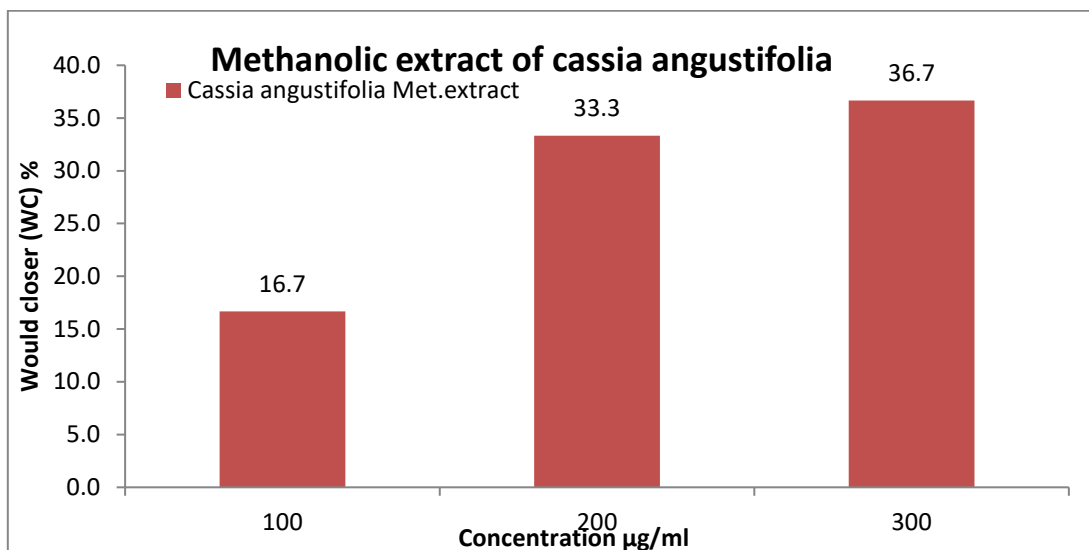


Fig: No:12. Methanol extract of cassia angustifolia flower

Table: No:5 Measurement of internal diameter(ID mm) and wound concentration percentage (WC%) on day five post-treatment with different concentrations of the leaves extracts of Tridax procumbens.

Table: No:5.1. Aqueous extract of tridax procumbens leaves

Aqueous extract	ID (mm)	WC%
100µg/ml	2.5 ± 0.09	16.667
200µg/ml	2.3 ± 0.04	23.333
300µg/ml	2.0 ± 0.16	33.333



Fig.No:13. 100µg/ml



Fig.No:14. 200µg/ml



Fig.No:15. 300µg/ml

The Aqueous extract of Tridax Procumbens leaves 100µg/ml, 200µg/ml, and 300µg/ml shows the result of 16.667%, 23.333% & 33.333% of wound closure activity.

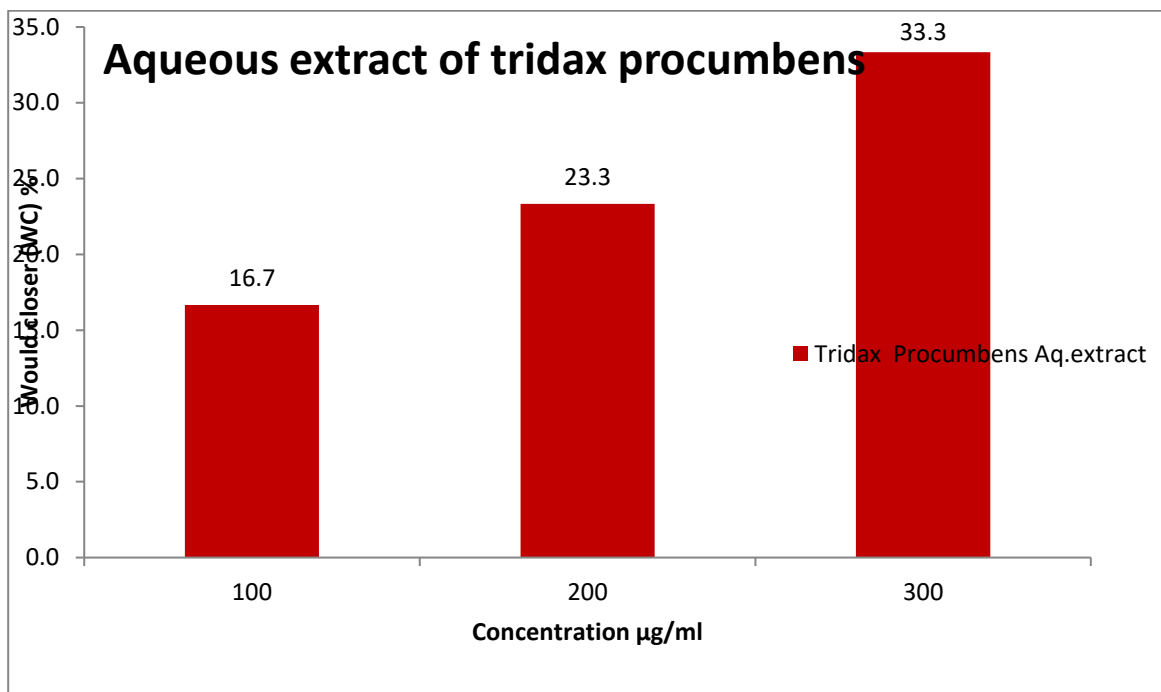


Fig: No:16. Aqueous extract of tridax procumbens leaves

Table: No:5.2. Methanolic extract of tridax procumbens leaves

Methanolic extract	ID	WC%
100µg/ml	2.0 ± 0.04	33.333
200µg/ml	1.9 ± 0.09	36.669
300µg/ml	1.7 ± 0.08	43.333



Fig.No:17. 100µg/ml



Fig.No:18. 200µg/ml



Fig.No:19. 300µg/ml

The Methanol extract of Tridax Procumbens leaves 100µg/ml, 200µg/ml, and 300µg/ml shows the result of 33.333%, 36.669% & 43.333% of wound closure activity

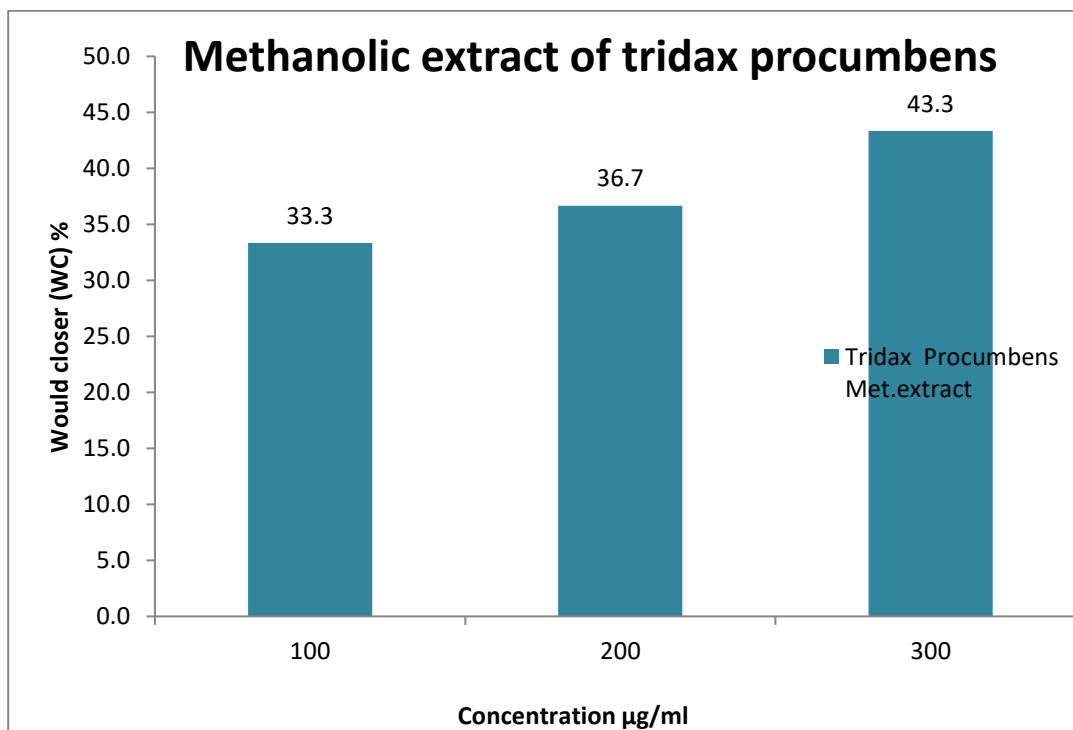


Fig: No:20. Methanolic extract of tridax procumbens leaves

Table: 6. Measurement of internal diameter(ID mm) and wound concentration percentage (WC%) on day five post-treatment with different concentrations of the Cassia Angustifolia Flowers & leaves extracts of Tridax procumbens aqueous extract.

Aqueous extract	ID (mm)	WC%
100µg/ml	2.2 ± 0.04	26.667
200µg/ml	1.9 ± 0.09	36.667
300µ/ml	1.4 ± 0.06	53.333
Methanolic extract	ID (mm)	WC%
100µg/ml	1.7 ± 0.16	43.333
200µg/ml	1.3 ± 0.05	56.666
300µg/ml	0.7 ± 0.09	76.667

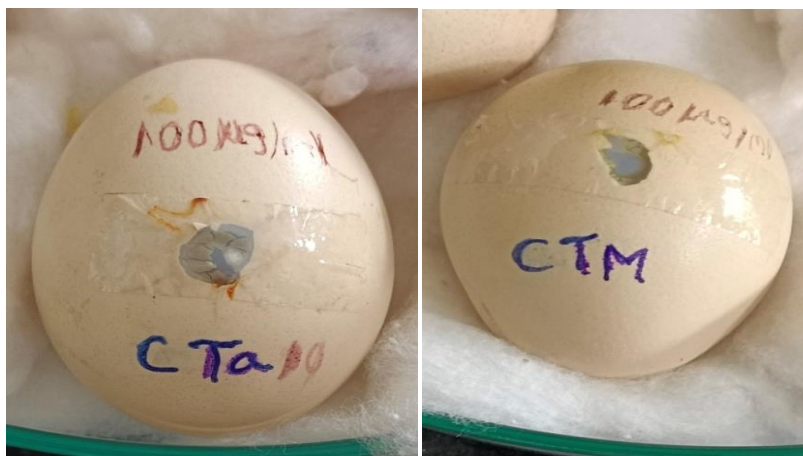


Fig.No:21. Comparison Between Cassia Angustifolia flower, Tridax Procumbens leaves Methanol Extract & Cassia Angustifolia, Tridax Procumbens Aqueous Extract at 100µg/ml

The methanol and aqueous extracts of cassia angustifolia flower, Tridax procumbens leaves 100µg/ml show the results of 43.333%, 26.666%.



Fig.No:22. Comparison Between Cassia Angustifolia flower, Tridax Procumbens leaves Methanol Extract & Cassia Angustifolia, Tridax Procumbens Aqueous Extract at 200µg/ml

The methanol and aqueous extracts of cassia angustifolia flower, Tridax procumbens leaves 200µg/ml show the results of 56.666%, 36.667%.



Fig.No:23. Comparison Between Cassia Angustifolia flower, Tridax Procumbens leaves Methanol Extract & Cassia Angustifolia, Tridax Procumbens Aqueous Extract at 300µg/ml

The methanol and aqueous extracts of cassia angustifolia flower, Tridax procumbens leaves 300µg/ml show the results of 76.667%, 53.333%.

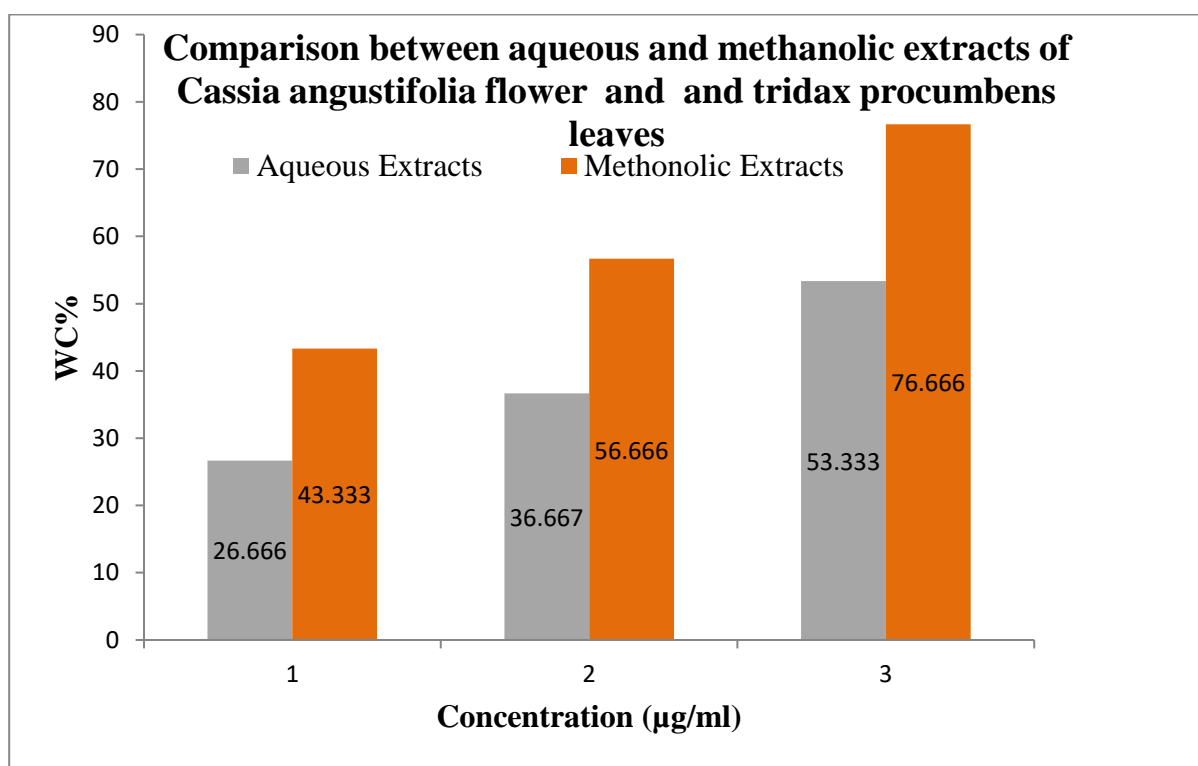


Fig.No:24. Comparison between aqueous extract of cassia angustifolia flower, tridax procumbens leaves and methanolic extract of cassia angustifolia flower, tridax procumbens leaves.

Table: No:7. Comparison between aqueous extract of cassia angustifolia flower, tridax procumbens leaves and methanolic extract of cassia angustifolia flower, tridax procumbens leaves.

CONC (µg/ml)	WC%	WC%
100 µg/ml	26.666 ± 0.6	43.333
200 µg/ml	36.667 ± 0.14	56.666
300 µg/ml	53.333 ± 0.19	76.666

CONCLUSION:

For preliminary screening of wound-healing phytochemicals, the established chick embryo wound model could serve as a substitute for the in-vivo animal models. The experiment was designed based on the expected wound concentration and angiogenic properties of substances with wound-healing potential. It was discovered that the flower extracts of *Cassia angustifolia* and *Tridax procumbens* possessed dose-dependent wound-healing properties.

The phytoconstituent profile derived from the phytochemical analysis revealed the plant's curative efficacy. Using the established model and knowledge of the target protein or other factors involved in the intricate healing pathway, additional molecular mechanisms underlying the activity could be determined.

Maximum wound closure was observed at 300 g/ml concentration, as determined by the Wound Closure Assay. At a concentration of 300g/ml, the methanol and aqueous extracts of *Cassia Angustifolia* demonstrated 36.66 percent and 26.66 percent wound closure, respectively, compared to the positive control, which showed 86.66 percent, and the negative control, which confirmed no significant wound closure. At 300g/ml concentration, methanol and aqueous *Tridax Procumbens* extracts exhibited wound concentrations of 43.33 and 33.33 percent, respectively.

Compared to the positive control, the wound concentration of the 1:1 ratio polyherbal preparation aqueous extract of *cassia angustifolia* flower, *tridax procumbens* leaves was 53.333 percent, and the wound concentration of the methanolic extract of *cassia angustifolia* flower, *tridax procumbens* leaves was 76.666 percent.

All concentrations of *cassia angustifolia* flower methanolic extract and *tridax procumbens* demonstrated effective wound-healing properties. In various treatments, angiogenesis was analyzed morphometrically by counting the number of blood vesicles. Compared to the saline controls, both extracts increased the number of blood vessels. The methanol extract was more angiogenic than the aqueous extract in terms of an increase in the number and thickness of blood vessels (1 to 25).

REFERENCES

1. K LednF, Villegas, Irma D, Femfindez, and Holger Maldonado. Evaluation of the wound healing activity of selected traditional medicinal plants from Peru. *Journal of Ethnopharmacology* 55(1997)193-200 ELSEVIER
2. Vinay kumar, Abdullah ahmed khan, k Nagarajan. animal models for the evaluation of wound healing activity. *International Bulletin of Drug Research*, 3(5):93-107, 2013
3. Dnyaneshwar D, Kokane, Rahul Y. Evaluation of wound healing activity of root of *Mimosa pudica*. *Journal of Ethnopharmacology* 124(2009) 311-315 ELSEVIER
4. KN. Chidambara Murthy, Vittal Reddy K, Jyothi M. Veigas and Uma D. Murthy. Study on Wound Healing Activity of *Punica granatum* Peel. *JOURNAL OF MEDICAL FOOD*, *JMedFood* 7(2)2004, 256-257
5. Reinaldo Ramos, Joao Pedro Silveira, Ana Cristina Rodrigues, Raquel Costa. Wound healing activity of the human antimicrobial peptide LL37. *Peptides* 32(2001)1469-1476 ELSEVIER
6. Kakali Saha, Pulok K. Mukherjee, J. Das, M. Pal, B. P. Saha. Wound healing activity of *Leucaslavandulaefolia* Rees. *Journal of Ethnopharmacology* 56 (1997)139-144 ELSEVIER
7. Vipin Kumar Garg and Sarvesh Kumar Paliwal. Wound healing activity of ethanolic and aqueous extract of *Ficus benghalensis*. *Journal of Advanced Pharmaceutical Technology & Research* 2011 Apr-Jun; 2(2):110-114
8. Wubante Demilew, Getnet Mequanint Adinew and Seyfe Asrade. Evaluation of the Wound Healing Activity of the Crude Extract of Leaves of *Acanthus polystachyus* Delile (Acanthaceae). *Hindawi Research Article Volume 2018*
9. Hemant Kumar Nagar, Amit Kumar Srivastava, Rajinish Srivastava, Madan Lal Kurmi, Harinarayan Sing Chandel, and Mahendra Sing Ranawat. Pharmacological Investigation of the Wound healing Activity of *Cestrum nocturnum* (L) Ointment in Wistar Albino Rats. *Hindawi Research*

Article Journal of Pharmaceutics Volume 2016, Article ID9249040, 8pages

10. Yaw D.Boakye,Christian Agyare,George P.Ayande,Nicholas Titiloye,Emmanuel A.Asiamah and Kwabena O.Danquah.Assessment of Wound Healing Properties of Medicinal Plants:The Case of Phyllanthus muellerianus.ORIGINAL RESEARCH article,21 August 2018.Frontiers in Pharmacology
11. Bezu Tekleyes,Solomon Assesfa Huluka,Kebede Wondu,Yohannes Tsegayie Wondmkun.Wound Healing Activity of 80% Methanol Leaf Extract of Zehneria scabra (L.f)Sond (Cucurbitaceae)in Mice. Journal of Experimental Pharmacology