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# Phytochemical screening and *in vitro* evaluation of wound healing activity of polyherbal preparation using chick embryo model

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**ABSTRACT: Background:** *Cassia angustifolia* and *Tridax procumbens* are plants reported for use in the siddha systems of medicines for treating various diseases. **Aim:** The study presented was an attempt to screen the methanol and aqueous extracts of the flowers and leaves of *C. angustifolia* and *T. procumbens* for wound healing potency. **Method:** The flower of *C. angustifolia* was extracted by Soxhlation using methanol and water as solvents. The leaf of *T. procumbens* plants was extracted by Soxhlation using ethanol and chloroform as solvents. The extracts of the two plants were tested for the presence of phytochemicals that are Carbohydrates, Glycosides, Alkaloids, Flavonoids, Tannins, Saponins, Steroids, Proteins, and Purins. The wound healing of the two plants was evaluated by using chick embryo wound model. **Results:** As evident from the experimental data the methanol extract showed good dose-dependent healing potency. The polyherbal methanol extract of dose 300 µg concentration showed increased wound concentration by 50 % compared to the negative control model in the chick embryo chorioallantoic membrane excision wound model. The results of polyherbal preparation are good compared with individual plants and control groups. **Conclusion:** The extract was also found to have been reported to be a reliable model and could be used as an alternative to animal models for preliminary screening of compounds with wound healing potency.

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## INTRODUCTION:

In Indian *Ayurveda* is one of the traditional medicinal systems. The basic logic *Ayurveda* is preventing unnecessary suffering and living a long healthy life. involves the use of Many natural elements has been used by the *Ayurveda* to eliminate the root cause of the disease and creating a healthy life [1].

In the history the herbal medicines have been extensively used world. As per the World Health Organization review, about 80 % of the word's

inhabitants used the traditional medicines for their health care [2].

The use of more than one herb in a medicinal preparation is considered as Polyherbal formulation (PHF). The PHF is found mainly in Ayurveda and other traditional medicinal system in India for the treatment of diseases [3].

Traditionally *C. angustifolia* are therapeutically used for the treatment of soothe coughs, wound and bronchitis**.** Essential oils of *C. angustifolia* possess antifungal and antibacterial activities [4].

Traditionally, *T. procumbens* L. is used to treat bronchial catarrh, diarrhea, wound healing hyperuricemia, oxidative stress, bacterial infection, dysentery and liver diseases [5].

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

## MATERIALS AND METHODS:

Sterile saline water and Marketed diclofenac tablet were procured from the local drug store. The eggs were procured from the local market. The methanol was purchased from S.D. Fine Chem, Mumbai.

### Collection, authentication and processing of Plant materials:

The fresh flower of C. *angustifolia* and leaves of *T. procumbens* were collected from the local areas of Kurnool. The collected plant materials were authenticated at the Botany Department of Government College at Kurnool. The collected plant material was shade dried for several weeks. The dried plants parts were crushed to make them in coarse form.

### Extraction:

The phytochemicals were extracted from the flowers of *C. angustifolia* by Soxhlation method using water and methanol as solvents. The leaves of *T. procumbens* was extracted by Soxhlation method by using water and methanol and chloroform as solvents [6,7].

### Phytochemical screening:

The water and methanol extract of *C. angustifolia* and ethanol and chloroform extract *T. procumbens* were subjected for the phytochemical screening for the detection of phytochemicals that are Carbohydrates, Reducing sugars, Saponins, Sennosides, Flavonoids, Alkaloids, Tannins, Amino acids, Steroids, and Glycosides. The phytochemical screening was done as per the standard procedure mentioned in the literature specification [8,9].

### Preparation of Polyherbal formula:

The polyherbal formulation was prepared in the 1:1 ratio of medicinal plant extract. About 0.1 g of *C. angustifolia* flower aqueous extract and *T. procumbens* leaves aqueous extract, 0.1 g of *C. angustifolia* methanol extract and 0.1 g of *T. procumbens* methanol extract was taken for the preparation of polyherbal.

### Wound healing activity study:

#### *Embryo collection*:

Fertilized white eggs was purchased from Kurnool. The outer surface of the embryos was cleaned with 75 % ethanol and incubated (SS 304 Laboratory Incubator-C-GEN, C-Gen Biotech, Mumbai) at 37 ºC throughout the study.

### Preparation of saturated filter disk for wound healing:

Whatman No. 1 filter paper was purchased from Madhu laboratories. Small disks were generated using a standard 5 mm hole puncher, sterilized by autoclaving (BR Biochem Wing Autoclave, BR Biochem, India) and stored for further use. The pre-sterilized filter disk was saturated with different concentrations of the crude extract from 100 to 300 µg/ml and the control solutions. Diclofenac sodium 50 (µg/ml) in 4 % methanol and sterile saline water were used as positive and negative controls respectively [10,11].

#### *Wound assay*:

All dissection tools used in the assay were sterilized using 75 % ethanol before use. The embryos were incubated for 11 days to allow good maturation of the chorioallantoic membrane. On the 12 day of incubation the outer shell was wiped with 75 % ethanol to sterilize the surface. Under aseptic conditions a tiny hole was made carefully in the egg shell with a needle and a small window of the was cracked open exposing the opaque inner shell membrane. About 0.5 to 1 ml sterile saline was added to the inner shell membrane to make it translucent. This layer was then peeled to visualize the CAM layer. The CAM layer was pulled gently by using sterile forceps and an excision wound of approximately 3 mm diameter was created in the CAM layer by using a small dissecting scissor. The drug saturated discs were then placed on the CAM of the embryos labelled with the corresponding concentrations and controls. The

window on the egg shell was covered with para film and eggs were returned to the incubator. Measurements of wound closure were made on alternative days up to day 5 of observation post wound healing. The wound contraction (WC) was calculated by using the following formula as mentioned in the equation 1 [12-18].

WC (%) = [(IWS × SWS)/ IWS] × 100 ….(1)

**RESULTS:**

The flower extract of C. *angustifolia and leaves of T. procumbens* was successfully prepared using the solvents water and methanol by Soxhlation method. The phytochemical screening study result (As given in Table 1 and 2) revealed that both the aqueous and methanol extract of *C. angustifolia* contains phytochemicals that were Carbohydrates, Reducing sugar, Saponins, Sennosides, Flavonoids, Alkaloids, Tannins, Amino acids, Steroids, and Glycosides (Table 1).

**Table 1. Phytochemicals present in flower extract of *C. angustifolia*.**

| Tests | Methanolic extract | Aqueous extract |
|---|---|---|
| Carbohydrates | + | + |
| Reducing sugars | + | + |
| Saponins | + | + |
| Sennosides | + | + |
| Flavonoids | + | + |
| Alkoloids | + | + |
| Tannins | + | + |
| Amino acids | + | + |
| Steroids | + | + |
| Glycosides | + | + |

**"+" Indicates Presence "_ "Indicates Absence.**

**Table 2. The phytochemicals present in the leaves extract of *T. procumbens*.**

| Tests | Methanol extract | Aqueous extract |
|---|---|---|
| Carbohydrates | + | + |
| Flavonoids | - | - |
| Alkoloids | + | + |
| Tannins | - | + |
| Amino acids | + | + |
| Steroids | + | + |
| Glycosides | - | - |
| Proteins | + | + |
| Purines | + | + |

**"+" Indicates Presence "_ "Indicates Absence.**

The aqueous extract of *T. procumbens* contains phytochemicals that were Carbohydrates, Alkaloids, Tannins, Amino acids, Steroids, Purines, and Proteins (Table 2). The methanol extract of *T. procumbens* contains phytochemicals that were Carbohydrates, Alkaloids, Tannins, Amino acids, Steroids, Tannins, Purines, and Proteins (Table 2).

The wound healing activity of *C. angustifolia* flower and *T. procumbens* leaves extract is given in Table 3 and Fig 1, 2, and 3.

**Table 3. Measurement of internal diameter (ID mm) and wound concentration percentage (WC %) on day 5 post treatment with tests, Positive Control (Marketed Diclofenac sodium 50 µg/ml), and Negative control (Saline).**

| Treatment | | ID (mm) | WC (%) |
|---|---|---|---|
| Positive Control (DS 50 µg/ml) | | 0.4±0.04 | 86.667 |
| Negative Control | | 3±0.01 | 0 |
| ***C. angustifolia*** | | | |
| AE | 100 µg/ml | 2.6±0.04 | 13.333 |
| | 200 µg/ml | 2.4±0.08 | 20 |
| | 300 µg/ml | 2.2±0.16 | 26.667 |
| ME | 100 µg/ml | 2.5±0.04 | 16.666 |
| | 200 µg/ml | 2.3±0.09 | 33.333 |
| | 300 µg/ml | 1.9±0.04 | 36.666 |
| ***T. procumbens*** | | | |
| AE | 100 µg/ml | 2.5±0.09 | 16.667 |
| | 200 µg/ml | 2.3±0.04 | 23.333 |
| | 300 µg/ml | 2.0±0.16 | 33.333 |
| ME | 100 µg/ml | 2.0±0.04 | 33.333 |
| | 200 µg/ml | 1.9±0.09 | 36.669 |
| | 300 µg/ml | 1.7±0.08 | 43.333 |

**AE, ME, ID, and WC are aqueous extract, methanol extract, internal diameter, and wound contraction.**

The 50 µg/ml of Diclofenac Sodium shows the result of 86.667 % of wound healing activity. Normal saline doesn't show any type activity. The aqueous extract of *C. angustifolia* flowers at doses of 100, 200, and 300 µg/ml shows the result of 13.33, 20, and 26.667 % of wound closure activity. The methanol extract of *C. angustifolia* flower at doses of 100, 200, and 300 µg/ml shows the result of 16.666, 33.33, and 36.66 % of wound closure activity. The aqueous extract of *T. procunbens* leaves at doses of 100, 200, and 300 µg/ml shows the result of 16.667, 23.333, and 33.333 % of wound closure activity. The methanol extract of *T. procunbens* leaves at doses of 100, 200, and 300 µg/ml shows 100, 200, and 300 µg/ml shows the result of 33.333, 36.669, 43.333% of wound closure activity.


**Fig 1. The wound healing activity of aqueous and methanol extract of *C. angustifolia* flower and T. *procumbens* leaves at various doses by chick embryo method.**

**a – Normal saline water; b – Diclofenac sodium (50 µg/ml); c, d, and e – *C. angustifolia* flower aqueous extract at doses of 100, 200, and 300 µg/ml; f, g, and h - *C. angustifolia* flower methanol extract at doses of 100, 200, and 300 µg/ml; I, j, and k – *T. procumbens* leave aqueous extract at doses of 100, 200, and 300 µg/ml; l, m, and n - *T. procumbens* leave methanol extract at doses of 100, 200, and 300 µg/ml.**

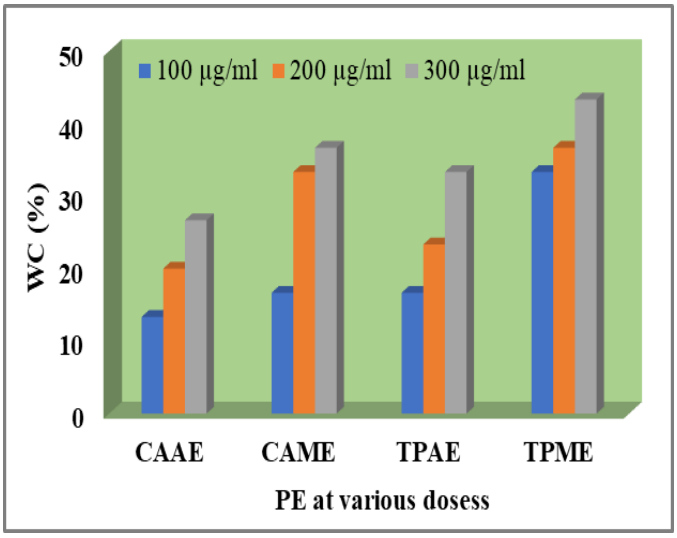


**Fig 2. Wound contraction (WC) data of various plant extract (PE) at various doses.**
**CAAE – *C. angustifolia* aqueous extract, CAME - *C. angustifolia* methanol extract, TPAE – *T. procumbens* aqueous extract, and TPME – *T. procumbens* methanol extract.**

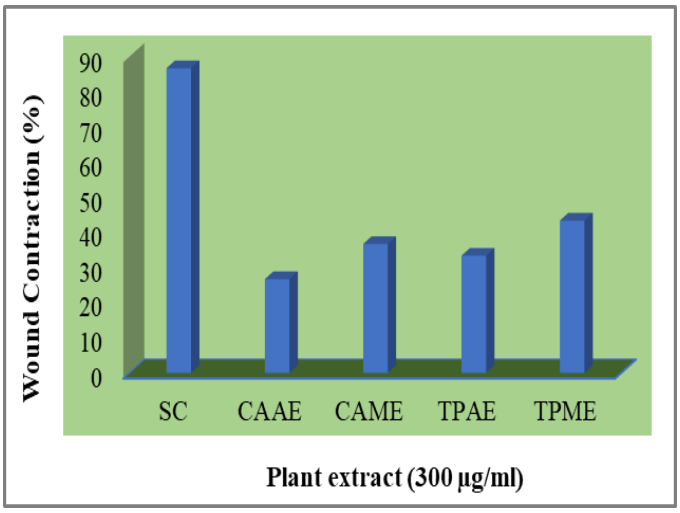


**Fig 3. The comparative Wound contraction (WC) data of standard control (SC) and various plant extract (PE) at dose of 300 µg/ml.**
**SC- Diclofenac sodium (50 µg/ml), CAAE – *C. angustifolia* aqueous extract, CAME - *C. angustifolia* methanol extract, TPAE – *T. procumbens* aqueous extract, and TPME – *T. procumbens* methanol extract.**

The polyherbal formulations was successfully prepared by using the aqueous and methanol flower extract of *C. angustifolia* and leaves extract of *T. procumbens*. The wound healing activity of polyherbal formulation is given in Table 4 and Fig 4 and 5. The polyherbal formulation containing the methanol and aqueous extracts of *C. angustifolia* flower and *T. procumbens* leaves at dose of 100 µg/ml show the results of 43.333 and 26.666 %. The polyherbal formulation containing the methanol and aqueous extracts of *C. angustifolia* flower and *T. procumbens* leaves at dose of 200 µg/ml show the results of 56.666 and 36.667 %. The polyherbal formulation containing the methanol and aqueous extracts of *C. angustifolia* flower and *T. procumbens* leaves at dose of 300 µg/ml show the results of 76.667 and 53.333 %.

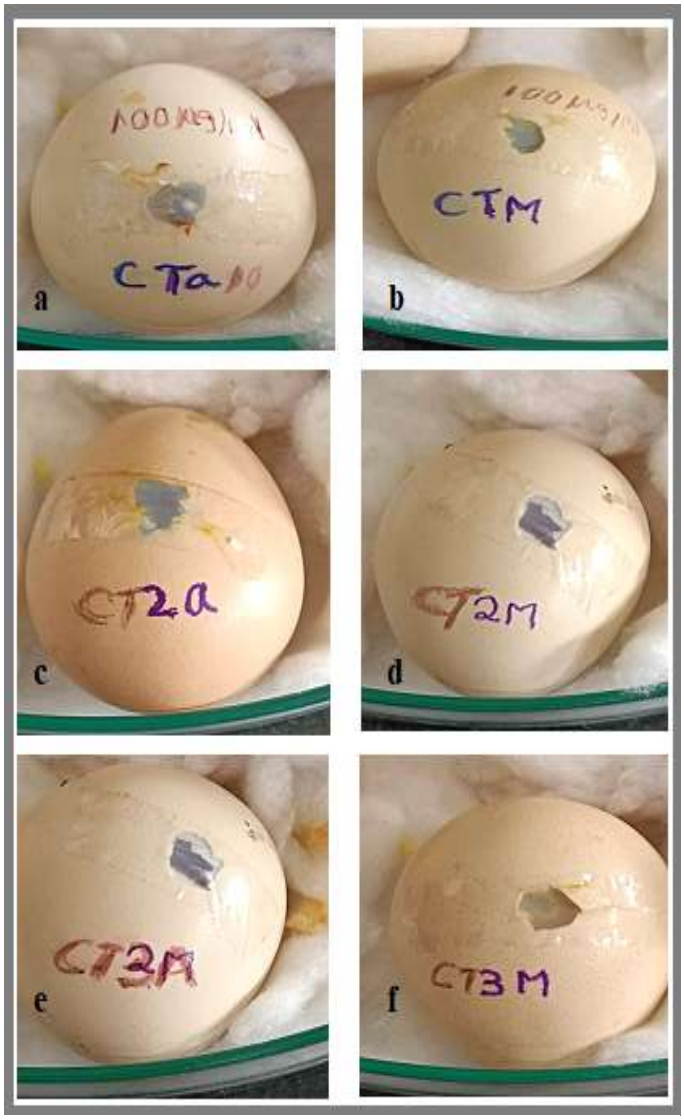


**Fig 4. The wound healing activity of polyherbal formulation of aqueous and methanol extract of *C. angustifolia* flower and T. *procumbens* leaves at various doses by chick embryo method.**
**a and b – Polyherbal formulation (1:1) of aqueous extract of *C. angustifolia* flower and *T. procumbens* leave at dose of 100 µg/ml. c and d - Polyherbal formulation (1:1) of aqueous extract of *C. angustifolia* flower and *T. procumbens* leave at dose of 200 µg/ml. e and f - Polyherbal formulation (1:1) of aqueous extract of *C. angustifolia* flower and *T. procumbens* leave at dose of 300 µg/ml.**

## DISCUSSIONS:

The established chick embryo wound model could serve as an alternative to the *in-vivo* animal model for preliminary screening of wound healing phytocompounds.

**Table 4. Measurement of internal diameter (ID mm) and wound concentration percentage (WC %) on day 5 post treatment with polyherbal formulations of *C. angustifolia* flower and *T. procumbens* leaves.**

| Treatment | | ID (mm) | WC (%) |
|---|---|---|---|
| AE 1:1 CA:TP | 100 µg/ml | 2.2 ± 0.04 | 26.667 |
| | 200 µg/ml | 1.9 ± 0.09 | 36.667 |
| | 300 µg/ml | 1.4 ± 0.06 | 53.333 |
| ME 1:1 CA:TP | 100 µg/ml | 1.7 ± 0.16 | 43.333 |
| | 200 µg/ml | 1.3 ± 0.05 | 56.666 |
| | 300 µg/ml | 0.7 ± 0.09 | 76.667 |

**AE, ME, ID, WC, CA, and TP are aqueous extract, methanol extract, internal diameter, wound contraction, *C. angustifolia*, and *T. procumbens*.**

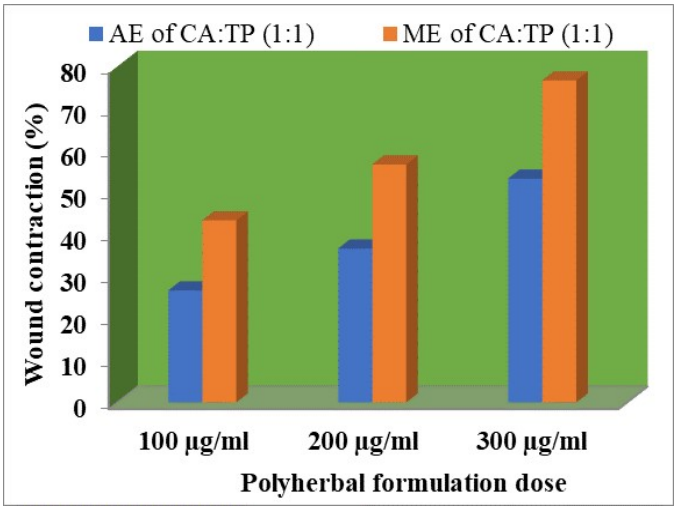


**Fig 5. The comparative wound healing activity of polyherbal formulation of aqueous and methanol extract of *C. angustifolia* flower and *T. procumbens* leaves at various doses by chick embryo method.**
**AE and ME of CA:TP – Aqueous and methanolic extract of *C. angustifolia* and *T. procumbens*.**

The experiment was designed based on the wound contraction and angiogenic traits expected to be possessed by compounds with wound healing potency. Of the two extracts of the *C. angustifolia* flowers and *T. procumbens* was found to possess excellent wound healing potency which was dose dependent. The healing potency of the plant was evident from the Phytoconstituents profile obtained from phytochemical analysis. Further molecular mechanism behind the activity could be determined using the established model with the knowledge of the target protein or other factors involved in the complex healing pathway.

The wound assay showed that percentage wound closure was dose dependent with the maximum being at 300 µg/ml concentration. At 300 µg/ml concentration the methanol and aqueous extracts of *C. angustifolia* showed 36.66 and 26.6 6% wound contraction compared to the positive control which showed 86.667 % and negative control which showed no significant wound closure. At 300 µg/ml concentration the methanol and aqueous extracts of *T. procumbens* showed 43.33 and 33.33 % wound contraction.

The 1:1 ratio polyherbal preparation aqueous extract of *C. angustifolia* flower, *T. procumben* leaves showed 53.333 % and methanolic extract of *C. angustifolia* flower, *T. procumben* leaves showed 76.666 % wound concentration compared to the positive control.

All concentrations were measured the methanolic extract of *C. angustifolia* flower and *T. procumbens* showed high wound healing potency.

Angiogenesis was morphometrically analyzed and counting the number of bloods vessels in various treatments. Both the extracts promoted an increase in number of blood vessels compared to saline controls. The methanol extract was more angiogenic in terms of increase in number and thickness of blood vessels than the aqueous extract.

## CONCLUSION:

From the above experimental study, it was significantly clear that the polyherbal formulation of *C. angustifolia* and *T. procumbent* showed potent wound healing activity. Thus, this polyherbal formulation could be an effective formulation for the treatment of any injury. But further study is required in in vivo manner to prove this effect mor significantly.

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