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SYNERGISTIC EFFECT OF *TRIDAX PROCUMBENS* AND *CURCUMA LONGA* AND THEIR THERAPEUTIC POTENTIAL AGAINST DIABETIC WOUND PATHOGENS IN TIRUPPUR DISTRICT, TAMILNADU

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Abstract

The present study has been undertaken to formulate and evaluate an herbal cream that contains a combination of *Tridax procumbens* and *Curcuma longa* extract. The selected medicinal plants *Tridax procumbens* and *Curcuma longa* are very promising species that produce secondary metabolites reported to have a variety of medicinal uses with specification to wound healing properties individually. Commercial antibiotics are ineffective due to multidrug resistance. The development of novel therapeutic bioactive potentials from ancient herbal medicines necessitates the betterment of human welfare becoming a need of an hour. In scenario of scientific findings, the development of Multi-drug resistance (MDR) of pathogens is overwhelmed by traditional herbal medicines due to their safety, cost-effectiveness and minimized. Ethanopharmacopia states that the two medicinal plants that were chosen, *Tridax procumbens* and *Curcuma longa*, are very promising species that contain secondary metabolites that are said to have a range of medicinal applications, with a focus on their unique roles in wound healing. The synergistic effect of these two plants has not been recorded to date. Hence, the present research envisioned the synergistic effect of best extracts from both the selected medicinal plants and their bioactive phytocompounds application against diabetic wound pathogens.

Keywords: Diabetic wound infection, Synergistic effect, Phytocompounds, Curcumin, Herbal cream.

I. INTRODUCTION

A wound is characterized by a disruption in the skin's cellular, anatomical, and functional epithelial integrity brought on by a physical, chemical, thermal, microbial, or immunological insult. This is followed by a disruption in the normal tissue beneath the skin's structure and function **[1, 2]**. Hemostasis, inflammation, proliferation, and remodeling are the four overlapping steps of the connective tissue repair process that make up the fundamental response in wound healing, according to Cianfarani *et al*., (2013) **[3]**. To finish the repair process during these stages, a variety of cells, growth factors, and cytokines must work together. Diabetes is one of the most common chronic diseases in both developed and developing countries **[4]**. Symptoms often include frequent urination, increased thirst, and increased appetite. If left untreated, diabetes can cause many complications **[5]**. Type 1diabetes is caused by autoimmune destruction of β-cells, while Type 2 diabetes is caused by insufficient correction for peripheral insulin resistance **[6, 7]**.

Medicinal herbs aid in debridement, disinfecting, and fostering an atmosphere that supports the body's natural healing process when it comes to wound care and management **[8]**. Plants play a unique and integral role in providing food, medicine, clothing, shelter, etc. Natural products have been extensively researched to discover new drugs **[9]**. There is a resurgence of interest in the use of medicinal plants to treat diabetic wounds since it is thought that their constituents are less hazardous and have fewer adverse effects than conventional treatments. This is because medicinal plants might have non-specific etiologies for diabetic wounds, making them a therapeutic option for therapy, particularly in environments with low resources **[10]**.

This study aims to create and assess an herbal cream that combines extracts from *Curcuma longa* and *Tridax procumbens*. The incorporation of other relevant medicinal components results in better indications for combinational therapy that provide insight into the likelihood of wound healing. There is currently no evidence of these two plants working in concert.

II. MATERIALS AND METHODS

2.1 Plant material collection extraction

The leaves of *Tridax procumbens* L- Asteraceae and the rhizomes of *Curcuma longa* L- Zingiberaceae were gathered from the Tiruppur area in Tamil Nadu, India. The two samples studied in the present investigation were authenticated in the Department of Botany, LRG Government Arts College for Women, Tiruppur. After being carefully collected, the leaves and rhizomes were immediately doused with ethanol, allowed to air dry in the shade, and then brought back to room temperature. In a blender, the dried leaves and rhizomes were ground into a powder. The powdered plant leaf and rhizome material were stored in sterile containers for subsequent use. 50 milliliters of diluted water, along with ethanol, acetone, methanol, and ethyl acetate as solvents, were combined with 2.5 grams of dried powder of *Tridax procumbens* and *Curcuma longa*. The combinations were then left to soak for a whole day. After passing through the Whatman No. 1 filter paper, the suspended particles were stored for two hours at 60°C in a water bath. For later usage, the dried crude extracts were kept in storage at 4°C.

2.2 Collection and identification of wound pathogens

Gram-positive (*Staphylococcus* sp. and *Streptococcus* sp.) and Gram-negative (*Escherichia coli*, *Pseudomonas* sp., and *Klebsiella* sp.) strains of wound isolates were obtained from KMCH, Sudha Hospital, and Government Hospital, Tiruppur was identified using a standard procedure, and the isolates were kept at 4°C in nutrient agar for additional confirmation research.

2.3 Antibiotic disc diffusion assay

The susceptibility testing technique used was the Kirby-Bauer disc diffusion method. The zone of inhibition's presence or absence was used to interpret the data **[11]**.

2.3.1 Antibacterial activity of plant extract

Plant extracts antimicrobial activity was tested against clinical pathogens, specifically *E. coli*, *Klebsiella* sp., *Pseudomonas* sp., *Staphylococcus* sp., and *Streptococcus* sp.

2.3.2 Preparation of synergistic mixture for antibacterial activity

Plant extracts were combined with other plant extracts to create synergistic combos. It was made using a blend of plant extracts in various ratios, such as 75:25, 25:75, and 50:50, respectively.

2.3.3 Determination of minimum inhibition concentration (MIC) microdilution

The lowest concentration of extract and synergistic extract at which it can exhibit bactericidal and bacteriostatic action was determined using minimum inhibition concentration. The lowest concentration of

the antibiotic at which the tested bacteria did not exhibit discernible growth was determined by taking the isolates minimum inhibitory concentrations, or MIC **[12]**.

2.4 Phytochemical analysis

The bioactive phytochemical screening in the plant extract was quantified and qualitatively estimated using conventional methodologies as outlined by Sani *et al*., 2007 **[13]**.

2.5 Antioxidant activity (Reducing assay)

After making 1 ml of extract in distilled water, 2.5 ml of phosphate buffer was added, and the mixture was left for 20 minutes. After adding 2.5 ml of 10% trichloroacetic (TCA) acid to the mixture, the mixture was centrifuged for 10 minutes at 3000 rpm. After mixing 1 ml of an aliquot of the supernatant with 2.5 ml of distilled water and 0.5 ml of 10% ferric chloride ($FeCl_3$), the absorbance was measured at 700 nanometers (nm). A higher reducing assay was inferred from an increase in absorbance.

2.6 Column Chromatography

Methanol was used to submerge air-dried *Curcuma longa* powder for the whole night. A small column (6 mm x 2 mm) chromatography was performed using 0.5 grams of ethanol extract. The packing material for the columns was silica gel (mesh 60–120). The solvent systems that were employed to elute the column were Hexane + Ethyl Acetate (95+5%), Acetone + Hexane (50+50%), Acetone + Ethyl Acetate (50+50%), Acetone + Methanol (50+50%), and Ethanol + Methanol (60+40%), (0.124, 0.33, 0.57, 0.755 and 0.908). Up to five fractions, 2 ml of each were collected after 10 ml of each series solvent was applied. Acetone, methanol, and acetic acid (75:08:50 µl) were used as a solvent mixture to dispense the collected fractions onto a TLC plate.

2.6.1 Phytochemical analysis using thin-layer chromatography (TLC)

Ethyl acetate, acetone, hexane, and ethanol were used to extract the most effective parts of *Curcuma longa*. A precoated silica gel plate was used to test 0.5 µl of the extracts at a concentration of 100 mg/ml for the presence of different component categories. The solvent systems saturated in the chamber used to generate the plate were acetone, methanol, and acetic acid (75:08:50µl).

2.6.1.1 P-anisaldehyde

P-anisaldehyde is a great all-purpose reagent. To create different hues on the plates, three or four drops of P-anisaldehyde were added.

2.6.1.2 Bromocresol green

The plates were coated with three to four drops of yellow-green carboxylic acid painted on a blue backdrop.

2.6.1.3 Test for ferric chloride

The plates were treated with three to four drops of ferric chloride reagent. The formation of a bluish-black color indicates the presence of phenols.

2.6.1.4 Iodine

Plates were shaken in an iodine chamber. Emerging dark areas are a sign of organic materials.

2.7 HERBAL CREAM FORMULATION

Aqueous Phase: Water dissolves any components that are soluble in it.
Oil Phase: In separate beakers, all components that were soluble in oil were combined at 75°C.
After carefully adding the aqueous phase to the oil phase and vigorously mixing the mixture, the mixture was homogenized for 30 minutes **[14]**.
The ingredients used in the herbal formulation were listed as follows,

S.NO	INGREDIENTS	QUANTITY
1	Herbal (*Tridax procumbens* and *Curcuma longa)*	1 ml
2	Glycerine	2 ml
3	Triethanolamine	3 ml
4	Liquid paraffin	7 ml
5	Ethanol	0.4 ml
6	Ethylenediaminetetraacetic acid	0.02 g
7	Sodium metabisulphite	0.02 g
8	Sodium benzoate	0.004 g
9	Water	Q.S (Quantity sufficient)

Table 1: List of ingredients of the formulation

2.8 EVALUATION OF THE CREAM

The prepared herbal cream was tested for microbiological growth, homogeneity and appearance, grittiness, spreadability, and pH.

2.8.1 Homogenicity

The herbal cream was placed in a container, and then its homogeneity was assessed by both tactile and optical inspection. Examining the hue, roughness, and pearlescence allowed for the determination of appearance **[15]**.

2.8.2 Grittiness

A light microscope was used to assess the herbal cream formulation to look for any particles **[16]**.

2.8.3 Spreadability

0.3 grams of the herbal cream was added to two slides, and after pressing the mixture to create a homogeneous thin layer, a 100-gram weight was added and left for five minutes. The spreadability was then determined using the formula

$$S = \frac{M \times L}{T}$$

Where M stands for the weight attached to the higher slide, L for the length of the glass slides, and T for the time required to separate the slides **[17]**.

2.8.4 Determination of pH

50 milliliters of distilled water were combined with 0.5 grams of the prepared herbal cream **[18]**. The pH meter was then used to measure the herbal cream pH at room temperature.

III. RESULTS AND DISCUSSION

Tridax procumbens (leaves) *and Curcuma longa* (rhizome) plant leaves and rhizome were collected and authenticated and the extract was prepared using different solvents such as Ethanol, Acetone, Methanol, Ethyl acetate, and Aqueous.

3.1 Identification of pathogens

The nine different strains of clinical wound pathogens were isolated from wound samples. All the strains were identified as *Klebsiella* sp, *Pseudomonas* sp, *Streptococcus* sp, and *E.coli* based on their morphological and biochemical characteristics.

The five strains of multi-drug resistant clinical isolates were collected and identified by selective plate method. That showed results of metallic sheen green colonies on the EMB plate, which indicates *E.coli*, yellow color colonies on the MSA plate, which indicates *Staphylococcus* sp, green colored colonies on the Cetrimide agar plate, which indicates *Pseudomonas* sp, pink mucoid colonies on the Mac Conkey agar plate, which indicates *Klebsiella* sp and white-greyish color colonies on the Blood agar plates which indicates *Streptococcus* sp.

3.2 Antibiotic-resistant assay

Antibiotic zone of inhibition for clinical wound pathogens (*Klebsiella* sp, *Staphylococcus* sp, *Pseudomonas* sp, *E. coli* and *Streptococcus* sp) was processed in 10 different antibiotics (OX- 1mcg, C-30mcg, RIF-5mcg, VA-30mcg, MRP-10mcg, OF-5mcg, TE-30mcg, GEN-10mcg, CZ-30mcg and CPM-30mcg). Oxacillin, Rifampicin, Cefazolin, and Cefopime showed the highest resistant activity using of showing resistance for a few strains. Then Tetracycline, Oflaxicin, and Gentamycin showed sensitivity only to E3 (23mm) and P2 (29mm) and showed the lowest level of resistant activity.

Studies conducted on MDR (Multi-drug resistant) among Gram-negative bacteria showed 90%, 90% and 60% of *E. coli* (E1, E2, and E3) resisted nine, nine, and six antimicrobial classes respectively. 70%, 50%, and 60% of *Pseudomonas* sp (P1, P2, and P3) resisted seven, five, and six antimicrobial classes respectively. 80% of *Klebsiella* sp, 40% of *Staphylococcus* sp, and 60% of *Streptococcus* sp resisted eight, four, and six antimicrobial classes respectively.

The clinical wound pathogens E1, P1, Sp, S1, and K1 showed the highest percentage of MDR index. In this study, the clinical wound pathogens showed resistance to different antibiotics, which showed that the bacterial strains had multidrug-resistant properties. These were selected for further process. From the results of the antimicrobial activity of antibiotics the strains E1, S1, P1, K1, and Sp were subjected to further process.

3.3 ANTIBACTERIAL ACTIVITY

3.3.1 Antibacterial activity of *Tridax procumbens* against wound pathogens

The *Tridax procumbens* plant extract outcomes are expressed as a millimeter-scale zone of inhibition (mm). Aqueous extract, ethanol, acetone, and ethyl acetate were evaluated against the clinical wound pathogens under investigation. The highest zone of inhibition against pathogens ranged from K1 (18 mm), S1 (18 mm), and Sp (19 mm) in the ethanol extract of *Tridax procumbens*. The extract made of ethyl acetate had the maximum efficacy against S1 (26 mm) and Sp (21 mm). The highest zone of inhibition against pathogens ranging from S1 (13mm) to Sp (14mm) was demonstrated by methanol extract. The largest zone of inhibition that the acetone extract exhibited against pathogens ranged as high as S1 (12 mm), while E1, K1, Sp, and P1 showed resistance.

3.3.2 Antibacterial activity of *Curcuma longa* against wound pathogens

The findings of plant extracts containing *Curcuma longa* are expressed as an inhibitory zone measured in millimeters (mm). Aqueous extract, methanol, ethanol, acetone, and ethyl acetate were evaluated against clinical wound pathogens. The highest zone of inhibition against pathogens ranged from E1 (20mm), P1 (22mm), K1 (22mm), S1 (18mm), and Sp (22mm) in the ethanol extract of *Curcuma longa*. Only in P1 (10mm) did the ethyl acetate extract exhibit the most activity, while E1, K1, S1, and Sp displayed resistance. The highest zone of inhibition against pathogens exhibited by methanol extract ranged from S1 (16mm) to Sp (25mm). The highest zone of inhibition of the acetone extract against pathogens up to S1 (23 mm) was observed.

3.3.3 Synergistic effect of Tridax procumbens extracts

The well diffusion method was used to determine different concentrations. The highest zone of inhibition against Streptococcus sp. (15 mm) was seen at 75:25 (Ethyl acetate: Ethanol) and 50:50 (Ethyl acetate: Ethanol) concentrations. Next, it was discovered that the concentration zone in the 25:75 (Ethanol: Ethyl Acetate) was 13 mm compared to *Klebsiella* sp.

3.3.4 Antimicrobial evaluation of the antimicrobial activity of various extracts of synergistic mixtures (*Tridax procumbens* with *Curcuma longa*)

The maximum zone of inhibition was found in the 50:50 concentrations, ethanol extract showed a zone of inhibition against *Staphylococcus* sp (13mm), *Klebsiella* sp (13mm), and *Streptococcus* sp (14mm). In ethyl acetate extract zone of inhibition was found against *Streptococcus* sp (13mm), *Klebsiella* sp (15mm), and *Staphylococcus* sp (13mm). Then in the acetone extract zone was found to be 13mm against *Streptococcus* sp.

3.3.5 Evaluation of Minimum inhibition concentration (MIC) of various extracts of *Tridax procumbens* and *Curcuma longa*

The ethanol extract of *Tridax procumbens* showed MIC values of strains like E1 (31.25mg/ml), P1 (31.25mg/ml), K1 (15.2mg/ml), S1 (7.81mg/ml), and Sp (15.62mg/ml). The ethyl acetate extract showed MIC values of strains like E1 (125mg/ml), P1 (31.25mg/ml), K1 (62.5mg/ml), S1 (7.81mg/ml), and Sp (15.62mg/ml). The ethanol extract of *Curcuma longa* showed MIC values of strains like E1 (31.25mg/ml), P1 (125mg/ml), K1 (125mg/ml), S1 (31.25mg/ml), and Sp (31.25mg/ml). The acetone extract showed MIC value of strains like E1 (31.25mg/ml), P1 (62.5mg/ml), K1 (62.5mg/ml), S1 (31.25mg/ml), and Sp (31.25mg/ml). There was no previous work regarding the MIC of *Tridax procumbens* and *Curcuma longa* Kumar *et al*., 2009 investigated the synergistic activity of plant extracts **[19]**. The synergistic ethanol extract of *Tridax procumbens* and *Curcuma longa* showed MIC value of strains like E1 (31.25mg/ml), P1 (125mg)/ml), K1 (125mg/ml), S1 (31.25mg/ml) and Sp (31.25mg/ml). Figure 1 presents the findings.

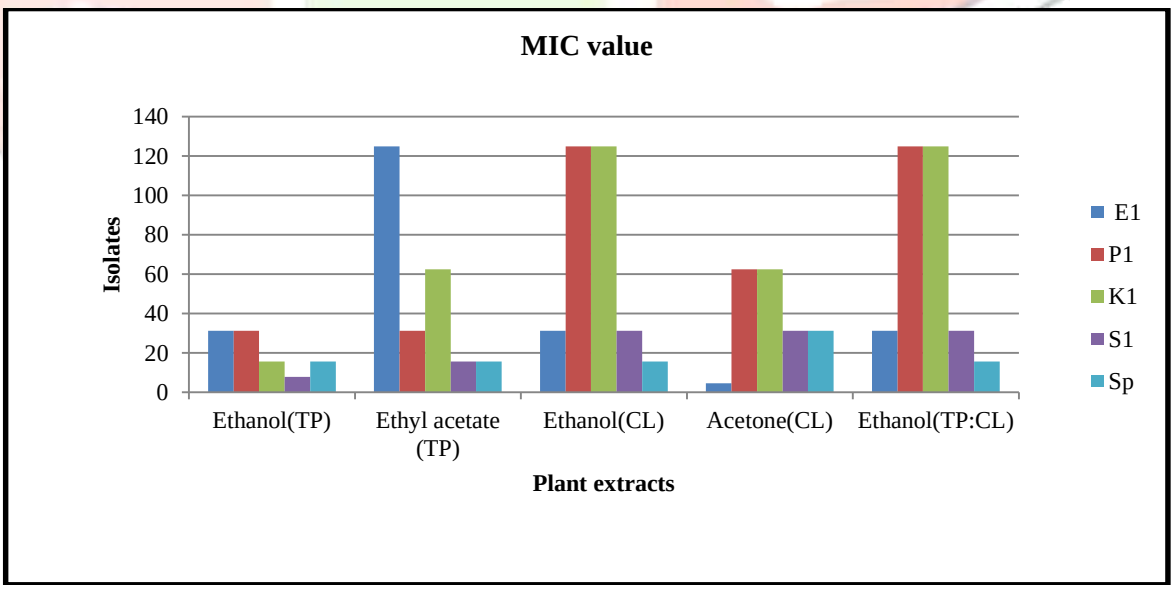

FIG 1: MIC Value of Plant extracts

3.4 PHYTOCHEMICAL ANALYSIS

3.4.1 Qualitative analysis of phytochemicals

The evaluation involved a qualitative phytochemical study of the leaves of *Tridax procumbens* and the rhizome of *Curcuma longa*. The majority of the phytoconstituents found in *Curcuma longa* and *Tridax procumbens* were proteins, alkaloids, flavonoids, saponins, terpenoids, phenols, and tannins. To determine whether bioactive components were present, a preliminary phytochemical screening of the leaves and rhizomes was carried out.

According to the findings, phenols, tannins, carbohydrates, terpenoids, alkaloids, flavonoids, glycosides, and proteins are present in *Tridax procumbens* leaf extract. Steroids and saponins were not found. The rhizome of *Curcuma longa* is rich in protein, carbohydrates, alkaloids, flavonoids, phenols, tannins, glycosides, terpenoids, and steroids. No saponins were found.
Based on the previous research the presence of Carbohydrate and protein in turmeric may be an indication, that it is a good source of Carbohydrate and protein **[20, 21]**. The presence of phytochemicals particularly Alkaloids and Flavonoids **[22]**, tannins, and Saponins account for the efficient antibacterial and medicinal activities of turmeric and other higher plants **[23, 24]**. They serve as defense mechanisms against many microorganisms, insects, and herbivores **[25]**.

3.4.2 Quantitative analysis of phytochemicals

Alkaloids, flavonoids, proteins, carbohydrates, ash, tannins, and phenols were all quantified and assessed. Quantitative Alkaloids were 8.8% (*Tridax procumbens*) and 14% (*Curcuma longa*); Flavonoids were 12% (*Tridax procumbens*) and 6.8% (*Curcuma longa*); Ash was 0.7% (*Tridax procumbens*) and 0.87% (*Curcuma longa*); Tannins were 0.02% (*Tridax procumbens*) and 0.20% (*Curcuma longa*); Phenols were 0.28% (*Tridax procumbens*) and 0.38% (*Curcuma longa*); Proteins were 10.97% (*Tridax procumbens*) and 10.4% (*Curcuma longa*); Carbohydrates were 10.4% (*Tridax procumbens*) and 12.7% (*Curcuma longa*); and Moisture was 0.91% (*Tridax procumbens*) and 0.59% (*Curcuma longa*).

3.5 Antioxidant activity

The antioxidant activity of *Tridax procumbens* and *Curcuma longa* ethanol extracts was examined as a fraction of their concentration. Three antioxidant assays revealed that the results were in an increased absorbance range with increased concentrations.

3.6 Column chromatography

The crude ethanol extract of *Curcuma longa* was subjected to column chromatographic for bioactive compound separation. A total of thirty fractions were obtained. In that six fractions were selected and subjected to evaluation of qualitative phytochemical analysis.

3.6.1 TLC analysis

Hence, the F5th, F7th, F9th, F12th, F17th, and F24th eluted fractions were selected to purify active molecules using thin-layer chromatography. P-anisaldehyde reagent shows a red or yellow color in selected three fractions (F5, F17, and F24) whereas reagent did not give a red or yellow color indicating there is no carbohydrate detected in selected fractions. Bromocresol green produced yellow-green on a blue background in selected three fractions (F7, F9, and F12) that indicate the presence of carboxylic acids. The formation of a bluish-black color indicates the presence of phenols in F7, F9, and F12 by the Ferric chloride test. The appearance of dark spots indicates organic compounds in F7, F9, and F12 by Iodine test.

3.7 FORMULATION OF HERBAL CREAM

The herbal cream was prepared using Carbopol 940 grade with the aqueous medium and neutralized using Triethanolamine. The herbal *Tridax procumbens* and *Curcuma longa* loaded cream was prepared by the slow mechanical mixing process.
The color of the cream was found intense bright yellow color. The designed herbal cream had a very smooth texture, according to the results. The pH was also maintained throughout the study which was found in the range of ±7.0. The formulated cream showed spreadability observed as ±13mm.

Fig 2: Formulation of herbal cream

3.8 EVALUATION OF HERBAL CREAM

The formulated herbal cream was evaluated by various standard parameters. The appearance of formulated herbal cream was judged by visual observation.

The formulated herbal cream was tested for the presence of microorganisms by culturing it with a Nutrient agar medium. There was no sign of pathogenic microbial growth after incubation for 24 hours at 24 hours at 37°C and it was comparable with the control.

IV. CONCLUSION

Our study concluded that the herbal cream with combination of *Tridax procumbens* and *Curcuma longa* extracts is one of the finest alternatives to synthetic cream. For the management and treatment of wounds, it is important to discover and produce plants or plant-derived compounds. Research in this area is now being conducted on several natural items. According to the results, the produced herbal cream's power to heal wounds, which is known to support the healing properties of these medicinal plants, maybe the reason for the observed efficacy. Comprehensive stability studies are necessary to improve the overall quality of the items.

Abbreviations and Acronyms

MDR- Multi-drug resistance, MIC- Minimum Inhibition Concentration, TCA- Trichloroacetic, NM- Nanometers (nm), TLC- Thin-layer chromatography, OX- Oxacillin, C- Chloramphenicol, RIF- Rifampicin, VA- Vancomycin, MRP- Meropenem, OF- Oflaxcin, TE-Tetracyclin, GEN- Gentamicin, CZ- Cefazolin and CPM- Cefopime, K1- *Klebsiella* sp, S1- *Staphylococcus* sp, P1- *Pseudomonas* sp, E1- *E. coli* and SP1- *Streptococcus* sp, TP-*Tridax procumbens*, CL- *Curcuma longa.*

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