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STUDIES ON PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITY IN LEAF AND FLOWER EXTRACTS OF *Tridax procumbens Linn*

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ABSTRACT

Tridax procumbens Linn belongs to the family Compositae. The extracts of *Tridax procumbens* have been reported to have various pharmacological, hepatoprotective, immunomodulatory and antiprotozoal effects. The leaves and flowers extracts of *Tridax procumbens* were screened phytochemically and the results revealed the presence of tannin, saponin, alkaloids, protein, diterpenes, phenol, cardial glycosides, amino acids and coumarins. The ethanol flower extract analysed by GC-MS for active biomolecules revealed the presence of bioactive molecules such as n-octocosane, Stearic acid, Myristic acid, Urcic acid, Oliec acid and n-Decyltetracosa. These compounds can be used as softening agents in cosmetics, pharamaceutics, and food industry as preservatives.

The antibacterial activity was carried out by agar well diffusion method against *Staphylococcus aureus, Salmonella typhi and Bacillus cereus.* The ethanol flower extract showed comparatively effective inhibition against the test organisms. Antifungal activity was carried out for 3 test fungi species (*Aspergillus niger, Penicillium Sp.* and *Rhizopus Sp.*). The efficiency of the extracts to increase the shelf life of tomatoes was performed and our results confirms that the ethanol flower extract of *Tridax procumbens* has extended shelf life of tomatoes when ethanol flower extract is coated on it. Our study reveals that the narrow spectrum preparations like leaves and flower extracts of *Tridax procumbens* can be considered as promising source of antimicrobial compound, which can be useful for successful therapy against multidrug resistant pathogens, without any side effects. It can further be recommended as a herbal food preservative which is nontoxic and more economic.

INTRODUCTION

With 'Herbal Renaissance' happening all over the globe, medicinal herbs are staging a phenomenal comeback. Ethno botanical information from India estimates that more than 6000 higher plant species forming about 40% of the higher plant diversity, are used in its codified and folk healthcare traditions (Ved and Goraya, 2007). India has a rich culture of medicinal herbs and species, which includes about more than 2000 and has a vast geographical area which have potential ability for Ayurvedic, Unani, Siddha traditional medicines but only few have been studied chemically and pharmacologically for their potential medicinal value.

In India, Ayurvedic System of medicine has existed for over four thousand years.Studying medicinal plants helps to understand plant toxicity and protect human and animals from natural poisons. Cultivation and preservation of medicinal plants protect biological diversity. Plants are directly used as medicines by a majority of cultures around the world, for example Chinese and Indian medicine (Sawant and Godghate, 2013). Plants have limitless ability to synthesize a vast array of bioactive compounds which possess some bioefficacy. These substances serve as plant defense mechanisms against predation of microbes, insects, herbivores. Phytochemicals from medicinal plants showing antimicrobial activities have the potential of filling this need, because their structure are different from those of the earlier studied microbial sources and therefore, their mode of action are likely to differ. *Tridax procumbens* is one such multifaceted weed available throughout the continent which can be used as a substitute for many herbs.

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Tridax procumbens

Vernacular names : English Coat Button and Tridax daisy, Spanish Cadillp Chisaca, French Herbe Caille, Chinese Kotobukigiku, Latin *Tridax* procumbens, Sanskrit Jayanti Veda, Hindi Ghamra, Marthi Dagadi Pala, Telgu Gaddi Chemanthi, Tamil Thata poodu, Malayam Chiravanak.

Tridax procumbens belongs to Family *Asteraceae*, known as 'Ghamra' and in English popularly called 'coat buttons' because of appearance of flowers. It has been extensively used in *Ayurvedic* system of medicine for various ailments and is dispensed for "Bhringraj" by some of the practitioners of Ayurveda. It is a common medicinal herb used by ethnomedical practitioners. It is best known as a wide spread weed and pest plant. It is native to the tropical Americas but it has introduced to tropical, subtropical, and mild temperature regions worldwide. It is widely distributed throughout India and found ubiquitously along roadsides, waste grounds, dikes, river banks, meadows and dunes (Sneha *et al.,* 2002).

Traditionally *Tridax procumbens* is found to possess significant medicinal properties against blood pressure, bronchial catarrh, malaria, dysentery, diarrhea, stomach ache, headache, wound healing. It also prevents hair fall and check hemorrhage from cuts and bruises (Ali *et al.,* 2000). Its flowers and leaf possess antiseptic, insecticidal and parasiticidal properties. The plant also shows various pharmacological activities like Immunomodulatory, Antidiabetic, Anti-hepatoxic and Anti-oxidant, Anti-inflammatory, Analgesic and marked depressant action on respiration (Ravikumar *et al.,* 2005). Leaf juice of *Tridax procumbens* was shown to depress wound contraction in experimental animals. Further it has been shown that extract of leaves also promotes wound healing in both normal and immune compromised (steroid treated) rats (Nia *et al.,* 2002).

Aqueous and alcoholic extract of leaves of *Tridax procumbens* showed a significant decrease in the blood glucose level in the model alloxan induced diabetes in rats (Vyas *et al.,* 2004). The ethanolic extract of the whole plant of *Tridax procumbens* showed significant antidiabetic activity against STZ – induced diabetes mellitus in the rats (Ramesh *et al., 2013)*.

Very few reports have focused on the immense potential of this plant which has antimicrobial, wound healing, anti-inflammatory and immunomodulatory properties (Suseela *et al.,* 2002; Taddei and Romero, 2000). However, there is paucity of studies on flower extracts of *Tridax procumbens*, its role as anti fungal agent and as a food preservative. Keeping this in view the present study has been undertaken to evaluate the different phytochemicals present in the chloroform, ethanol and aqueous extracts of the flower and leaves of the *Tridax procumbens*. Further, this study focuses on antimicrobial activities of extracts from *Tridax procumbens* against general food spoilage and human pathogens so that new herbal food preservative may be developed.

MATERIALS AND METHODS

Collection of Plant Material: The leaves of *Tridax procumbens* plant were collected from campus of Karnatak University, Dharwad. The collected plant material after ridding them of dirt, the leaves and flowers were shade dried and powdered using grinder and sieved to get fine powders. The fine powders were then subjected to successive extractions by chloroform, ethanol, methanol and aqueous extracts. These extracts were concentrated using rotary flash evaporator and preserved in a bottle until further use.

Phytochemical analysis: Different types of tests have been performed for the presence of fallowing phytochemical constituents: Steroid, Tannin, Saponin, Anthocyanin, Coumarin, Emodins, Alkaloids, Proteins, Aminoacids, Diterpenes, Pytosterol, Phenol, Phlobatannins, Leucoanthocyanin, Cardial Glycosides, Flavonoids. Phytochemical tests were carried out by adopting standard procedures (Ikewuchi *et al.,* 2009).

Steroid: 1ml extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 acid was added from the side of test tube. The upper layer turns red and H_2SO_4 layer showed yellow with green fluorescence. This indicates the presence of steroid.

Tannin

a. 2ml extract was added to 1% lead acetate a yellowish precipitate indicates the presence of tannins.
b. 4ml extract was treated with 4ml $Fecl_3$ formation green colour indicates that presence of condensed tannin.

Saponin: 5ml extract was mixed with 20 mi of distilled water then agitated in graduated cylinder for 15minutes formation of foam indicates saponin.

Anthocyanin: 2ml of aqueous extract is added to 2ml of 2N HCl and NH_3, the appearance of pink red turns blue violet indicates presence of anthocyanin.

Coumarin: 3ml of 10% NaOH was added to 2ml of aqueous extract formation of yellow colour indicates coumarin.

Emodins: 2ml of NH_4OH and 3ml of benzene was added to extract appearance of red colour indicates presence of emodins.

Alkaloids: A quantity (3ml) of concentrated extract was taken into a test tube and 1ml HCl was added the mixture was heated gently for 20min cooled and filter, the filtertate was used for fallowing test:

a. Wagner test: Filterate was treated with Wagner's reagent; formation of brown reddish precipitate indicates presence of alkaloids.

b. Hager's test: Filterate was treated with Hager's reagent, presence of alkaloid confirmed by the yellow colour precipitate.

Proteins

Xanthoproteic test: Extract were treated with few drops of concentrated HNO_3 formation yellow colour indicates the presence of proteins.

Amino acids

Ninhydrin test: To the 2ml extract 2ml of ninhydrin reagent was added and boil for few minutes, formation of blue colour indicates presence of amino acid.

Diterpenes: Copper acetate test: Extract were dissolved in water and treated with 10drops of copper acetate solution, formation of emerald green colour indicates presence of diterpens.

Pytosterol*:* Salkowski's test: Extract was treated with chloroform and filtered. The filterate was treated with few drops of concentrated H_2SO_4 shake it well, allow standing, appearance of golden red colour indicates positive test.

Phenol

Ferric Chloride test: Test extract were treated with 4 drops of Alcoholic $FeCl_3$ solution. Formation of bluish black colour indicates the presence of Phenol.

Phlobatannins: Deposition of red precipitate when aqueous extract of each plant sample is boiled with 1% Aqueous HCl was taken as evidence for presence of phlobatannins.

Leucoanthocyanin: 5ml of isoamyl alcohol added to 5ml of aqueous extract, upper layer appear red in colour indicates the presence of leucoanthocyanin.

Cardial Glycosides

Keller-Killani test: Plant extract treated with 2ml glacial acetic acid conaining a drop of $FeCl_3$. A brown colour ring indicates the presence of positive test.

Flavonoids

a. Alkaline reagent test: Extract was treated with 10% NaOH solution, formation of intense
b. yellow colour ring indicates the presence of flavonoid.
c. NH_4OH test: 3ml of extract were treated with 10% NH_4OH solution development of yellow
d. fluorescence indicates positive test.
e. Mg turning test: Extract were treated with Mg turning and conc.HCl to this solution add 5ml
f. of 95% ethanol, formation of crimson red colour indicates presence of flavonoid.
g. Zn test: 2mlextract were treated with Zn dust and conc.HCl development of red colour indicates the presence of flavonoids.

Antimicrobial activity: Pathogenic bacteria like *Staphylococcus aureus, Bacillus cereus, Salmonella typhi* were procured from Department of Biotechnology and Microbiology Karnatak University Dharwad. Food spoilage causing fungi like *Rhizopus Sp.* is procured from Department of Biotechnology and Microbiology Karnatak University Dharwad. *Penicillium Sp.* and *Aspergillus niger* were collected from Department of Botany Karnatak University, Dharwad

Antibacterial activity*:* Mueller Hinton agar NO:2 (HIMEDIA) was employed for antibacterial activity by Agar well diffusion method. The antibacterial activity was carried out by employing 24 hour culture of *Staphylococcous aureus, Salmonella typhi* and *Bacillus cereus.* Activity of ethanol, chloroform and aqueous extracts of both leaves and flowers were tested separately using agar well diffusion method. The inoculated plates with plant extract were incubated at 37^0 C for 24 hours.

Minimum inhibitory concentration: Minimum inhibitory concentration (MIC) was determined which was showing good activity against test pathogens (Ethanol flower extract) by agar well diffusion method. To check the MIC of extract, agar well diffusion method was carried out by taking the different concentration of plant extracts (30µg/ml, 40µg/ml, 50µg/ml, 60µg/ml, 70µg/ml, 80µg/ml) and by taking standard antibiotics(Ceftriaxone, cefotaxime, ampicillin, ofloxacin) with their respective standard concentration (Jindal *et al.,* 2013).

Petriplates containing 20ml of Muller Hinton medium were seeded with 24 hour culture of bacterial strains. A well of 6mm diameter was made using sterile cork borer. The plant extract was dissolved in mother solvent then the extract was added to the wells. The standard antibiotics were taken as a positive control. The plates were then incubated at 37ºC for 24 hours. The antibacterial activity was assayed by measuring the diameter of the zone formed around the well.

Determination of antifungal activity using poison food technique: The principle involved in this technique is to 'poison' the nutrient medium with a fungi toxicant here using our plant extract sample, and then allow a test fungus to grow on such a medium (Jindal and Kumar., 2012). Potato dextrose agar was prepared and sterilized. Requisite quantity of plant extract was added to the medium (at Luke warm stage) so as to get a desired concentration of 5% and 10%. Then the plant extract was mixed thoroughly by stirring, poured into sterilized petriplates and allowed to solidify. Cut small discs of (3mm) test fungi with sterile cork borer and aseptically transfered to the center of a petriplate containing medium with plant extract and incubate at $28^0 \pm 2^0$ C in the incubator. Similar method is followed for media without plant extract which is used as a control. Measure the fungal colony diameter when the colony growth in control plates is full. The colony diameter, compared with control.

Percent of growth inhibition over control is calculated by the following formula.

$$I=100(C-T)/C$$

Where, I = Inhibition percentage, C = Growth in control, T = Growth in treatment

The growth of fungal colony in treated plates is determined by excluding 3mm fungal inoculation discs from measured diameter of the colony. The same method is also carried out for pure mother solvents.

Gas chromatography and mass spectroscopy analysis of the sample (GC-MS): To know the probable bioactive compounds of the plant extract which showed good antimicrobial activity (Ethanol flower extract) is subjected to GC-MS analysis. The GC -MS analysis is performed in Univesity Science Instrumentation Centre (USIC) of Karnatak University, Dharwad. GC - MS analysis is performed using CARBOWX capillary column and Helium as a carrier gas to quantify the probable major phytochemical in the extract. The identification is based on comparison of their mass spectra and retention indices.

Application of selected plant extract on Tomatoes: The thought behind this experiment was to check for the application of *Tridax procumbens* extract as a biocontrol agent. To begin with we chose tomatoes as a model. The plant extract which showed good antibacterial activity (Ethanol flower extract) was used to apply on tomatoes. The concentration is prepared according to the MIC against tested pathogens and applied on tomatoes to check the shelf life. The tomatoes were treated with the flower extracts of *Tridax procumbens* and incubated for a period of fifteen days along with positive and negative control. They were observed for physical changes like colour , texture etc at 5 days of intervals. The physical changes were observed by comparing negative control (Untreated), positive control (Saline treated) and treated (Ethanol flower extract) tomatoes.

RESULTS AND DISCUSSION

The phytochemical analysis of leaf extract of *Tridax procumbens* revealed that the chloroform extract showed the presence of tannin, saponin, alkaloids, protein, diterpenes, phenol, cardial glycosides and slightly flavonoids. The ethanol extract showed the presence of tannin, saponin, coumarins, alkaloids, proteins, amino acids, diterpenes, cardial glycosides and moderately flavonoids and the aqueous extract showed the presence of steroids, coumarins, alkaloids, phenols, cardial glycoside**s,** and moderately flavonoids.The chloroform extract of flower of *Tridax procumbens* showed the presence of tannin, saponin, alkaloids, protein, diterpenes, cardial glycosides and moderately flavonoids. The ethanol extract showed the presence of steroids, tannin, saponin, coumarins, alkaloids, proteins, amino acids, phytosterol, and strongly flavonoids while the aqueous extract showed the presence of tannin, coumarin, alkaloids, cardial glycosides, and moderately flavonoids.

Similar results of phytochemical screening were also reported that revealed the presence of alkaloids, carotenoids, flavonoids and tannins, indicating that it is richly endowed with carotenoids and saponins. The proximate profile shows that the plant is rich in sodium, potassium and calcium (Sneha *et al*., 2010). The chemical constituents of the plant showed that its leaves contain various alkaloids, flavonoids, carotenoids, fumaric acid etc (Surendra *et al.,* 2004).

Table 1 Phytochemical analysis of leaf and flower extracts of *Tridax procumbens*
(+ = Present; - =Absent)

Phytochemical Tests	Leaf			Flower		
	Chloroform extract	Ethanol extract	Aqueous extract	Chloroform extract	Ethanol extract	Aqueous extract
1. steroids	_	_	+	_	+	_
2. Tannin i. Lead acetate test	+	+	_	+	+	+
ii. Ferric chloride test	+	+	_	+	+	_
3. Saponin	+	+	_	+	+	_
4. Anthocyanin	_	_	_	_	_	_
5. Coumarin	_	+	+	_	+	+
6. Emodins	_	_	_	_	_	_
7. Alkaloids i. Wagner test	+	+	+	+	+	+
ii. Hager's test	+	+	+	+	+	+
8. Proteins (Xanthoproteic test)	+	+	_	+	+	_
9. Amino acids (Ninhydrin test)	_	+	_	_	+	_
10. Diterpenes test (Copper acetate test)	+	+	_	+	_	_
11.Phytosterol (Salkowski's test)	_	_	_	+	+	_
12. Phenol (Ferric chloride test)	+	_	+	_	+	_
13. Phlobatannins	_	_	_	_	_	_
14. Leucoanthocyanin	_	_	_	_	_	_
15. Cardial glycosides (Keller-Killani test)	+	+	+	+	_	+
16. Flavonoid i. Alkaline reagent test	-	+	+	+	+	+
ii. NH4OH test	+	+	-	+	+	+
iii. Mg turning test	-	-	-	-	-	-
iv Zn test	-	-	+	+	-	-

Table 2 The antibacterial effect of ethanol flower extract

Sl no	Organism	Ethanol flower extract (µg/ml)						Ceftriaxone	Cefotaxime	Ampicillin	Ofloxacin
		Zone of inhibition (mm)									
		30	40	50	60	70	80	Standard concentration			
1	***Staphylococcus aureus***	8	9	12	14	15	16	14	16	17	19
2	***Bacillus cereus***	15	16	20	21	22	30	15	20	15	20
3	***Salmonella typhi***	6	7	7	8	10	14	20	18	12	26

Dhanabalan *et al*., (2009) also revealed the presence of alkaloids, tannin, saponin, steroid, phlobatannin, terpenoids, flavonoids and cardiac glycosides form the methanolic extract of leaves of *T. procumbens Linn*.

Antibacterial activity

Table 3 MIC of ethanol flower extract

Sl No	Name of the Organism	MIC (µg/ml)
1	*Staphylococcus aureus*	50
2	*Bacillus cereus*	30
3	*Salmonella typhi*	70

Fig 2 Zone of inhibition of *Staphylococcus aureus*

Fig 3 Zone of inhibition of *Bacillus cereus*

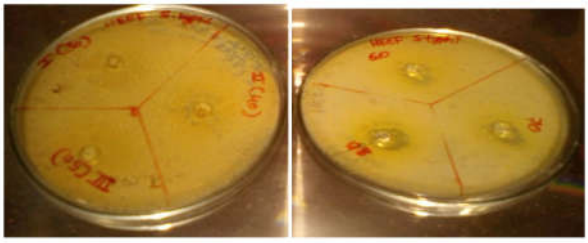


Fig 4 Zone of inhibition *Salmonella typhi*

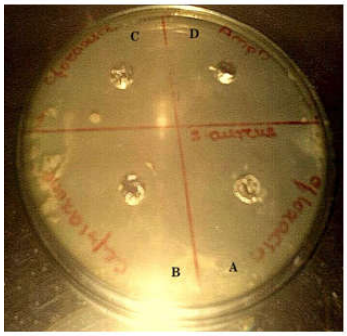


Fig 5 Antibiotic sensitivity of *Staphylococcus aureus*

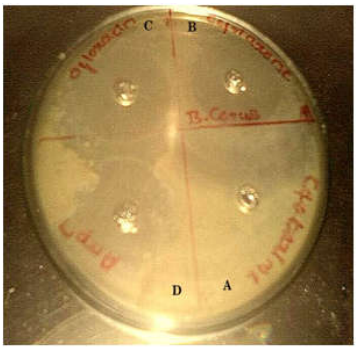


Fig 6 Antibiotic sensitivity of *Bacillus cereus*

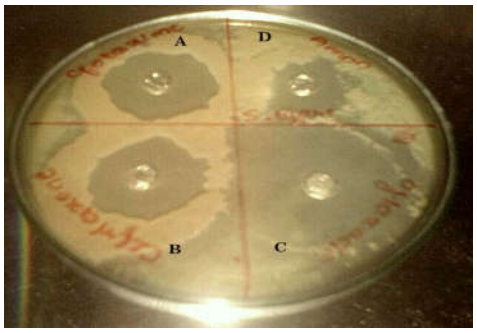


Fig 7 Antibiotic sensitivity of *Salmonella typhi*

(A= Cefotaxime; B = Ceftriaxone; C = Ofloxacin; D = Ampicillin)

The chloroform, ethanol and aqueous extract of both leaves and flower of *Tridax procumbens* was investigated for their antibacterial activity. It was observed that ethanol flower extract showing good antibacterial activity against *Staphylococcus aureus, Bacillus cereus* and *Salmonella typhi*. Further, the ethanol flower extract is selected for determination of Minimum Inhibitory Concentration (MIC).

The antibacterial activity of ethanol flower extract at different concentration is compared with standard antibiotics like, Cefotaxime, Ceftriaxone, Ofloxacin and Ampicillin with their standard concentrations. The comparison is based on the zone formation around the well (Table-2). The result confirmed that ethanol flower extract is showing maximum antibacterial activity against *Bacillus cereus* > *Staphylococcus aureus* > *Salmonella typhi*. It was found that MIC for *Staphylococcus aureus* is 50µg/ml (Fig-2), for *Bacillus cereus* it is 30µg/ml (Fig-3) and for *Salmonella typhi* it is 70µg/ml (Fig-3) (Table-3).

Antifungal activity

The antifungal activity of chloroform, ethanol and aqueous extract of both leaves and flower of *Tridax procumbens* was carried out at two different concentrations (5% and10%) by poisoned food method. The inhibition of growth of fungi by plant extract is compared with control plates (Fig- 8, 9 and 10).

Table 3 The growth inhibition of fungi by pure solvent (RG = Radial growth; inh (%) = Percentage of inhibition)

S.no	Solvent	*Aspergillus niger*		*Penicillium Sp.*		*Rhizopus Sp.*	
		RG (mm)	Inh (%)	RG (mm)	Inh (%)	RG (mm)	Inh (%)
1	**Chloroform**	47	47.77	44	51.11	53	41.11
2	**Ethanol**	40	55.55	43	52.22	45	50.00
3	**Aqueous**	89	1.11	88	2.22	90	0.00

Fig 8 *Aspergillus niger* (Control) **Fig 9** *Rhizopus Sp.*(Control) **Fig 10** *Penicillium Sp.* (Control)

Fig 11 Chloroform leaf extract (5%) inhibiting growth of *Aspergillus niger* **Fig 12** Chloroform leaf extract (5%) inhibiting growth of *Rhizopus* **Fig 13** Chloroform leaf extract (5%) inhibiting growth of *Penicillium*

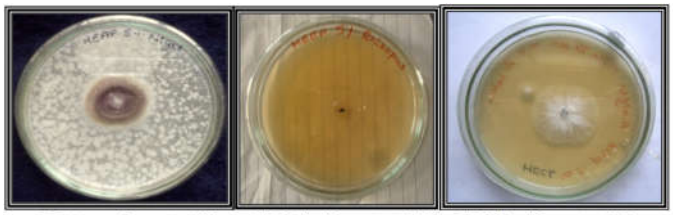


Fig 14 Aqueous flower extract (5%) inhibiting growth of *Aspergillus niger* **Fig 15** Ethanol flower extract (5%) inhibiting growth of *Rhizopous Sp.* **Fig 16** Ethanol flower extract (5%) inhibiting growth of *Penicillium Sp.*

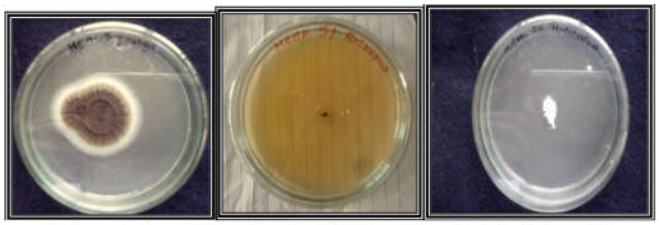


Fig 17 Aqueous leaf extract (5%) inhibiting growth of *Aspergillus niger* **Fig 18** Ethanol leaf extract (5%) inhibiting growth of *Rhizopous Sp.* **Fig 19** Ethanol leaf extract (5%) inhibiting growth of *Penicillium Sp.*

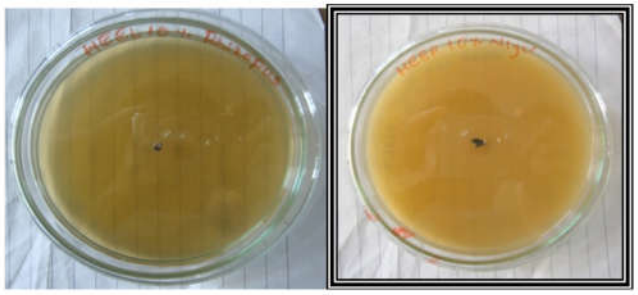


Fig 20 Aqueous leaf extract (10%) inhibiting growth of *Aspergillus niger* **Fig 21** Aqueous leaf extract (10%) inhibiting growth of *Rhizopus Sp.*

Fig 22 Aqueous leaf extract (10%) inhibiting growth of *Penicillium Sp.*

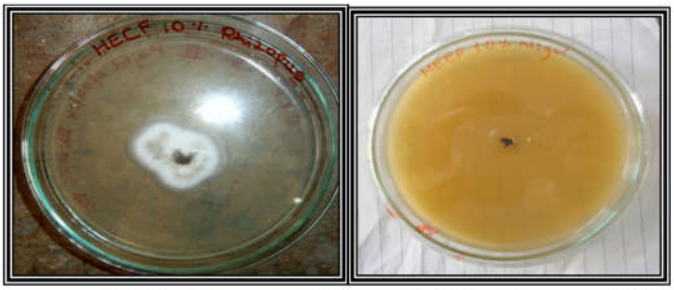


Fig 23 Ethanol flower extract (10%) inhibiting growth of *Aspergillus niger* **Fig 24** Ethanol flower extract (10%) inhibiting growth of *Rhizopous Sp.*

Fig 25 Ethanol flower extract (10%) inhibiting growth of *Pennicilium*

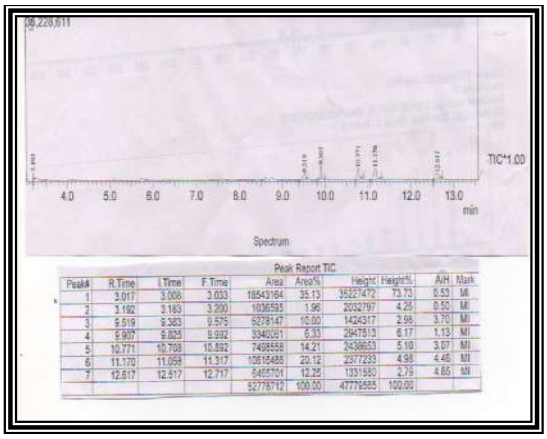
Table 4 Antifungal activity of different extract (5%) of *Tidax procumbens* (RG = Radial growth; inh (%) = Percentage of inhibition)

S.no	Extract (5%)	*Aspergillus niger*		*Penicillium Sp.*		*Rhizopus Sp.*	
		RG (mm)	Inh (%)	RG (mm)	Inh (%)	RG (mm)	Inh (%)
1	Chloroform leaf extract	10	88.88	16	82.22	15	83.33
2	Ethanol leaf extract	1	98.88	14	84.44	10	88.88
3	Aqueous leaf extract	07	92.22	3	96.64	7	92.22
4	Chloroform flower extract	15	83.33	14	84.44	5	94.44
5	Ethanol flower extract	9	90.00	16	82.22	1	98.88
6	Aqueous flower extract	6	93.33	14	84.44	16	82.22

10% extract: The antifungal activity of 6 extracts of both leaves and flower (chloroform, ethanol and aqueous) at 10% against *Aspergillus niger, Penicillium Sp.* and *Rhizopus Sp.* is represented in table-5. The maximum inhibition of growth of fungi by each plant extract is as follows.

The chloroform leaf extract inhibited 94.44% growth of *Aspergillus niger* (Fig-18). Ethanol leaf extract inhibited 98.88% growth of *Rhizopus Sp.* (Fig-19). Aqueous leaf extract inhibited 91.11% growth of *Aspergillus niger* (Fig-20). Chloroform flower extract inhibited 96.66% growth of *Rhizopus Sp.* (Fig-21). Ethanol flower extract inhibited 98.88% growth of *Aspergillus niger* (Fig-22). Aqueous flower extract inhibited 97.77% growth of *Aspergillus niger* (Fig-23).
The antifungal activity of pure mother solvent is represented in table-6.

GC-MS Analysis: The ethanol flower sample was analysed in GC – MS. Based on retention time in the chromatogram (Graph:1) the compounds analyzed using CHEM – DRAW software were identified as n- octocosane, Stearic acid, Myristic acid, Urcic acid, Oliec acid and n-Decyltetracosa (Table 5).



Graph 1 GC-MS Analysis of ethanol flower extracts

Table 5 Probable compounds analyzed by GC-MS in ethanolic flower extract of *Tridax procumbens*

Sl. No	Compounds analyzed by GCMS
1	n- Octacosane
2	Stearic acid
3	Myristic acid
4	Erucic acid
5	Oliec acid
6	n- Decyltetracosa

Effect of ethanol flower extract in defense against natural rottening of tomatoes Application of Ethanol flower extract on Tomatoes

a. On the 1st day the results were observed (Fig -26 A).
b. On 5th day of incubation no physical changes was observed in negative control, positive control and treated tomatoes (Fig -26 B)
c. On 10th day of incubation there is softening of texture & change in color was observed in negative control where as positive control & treated tomatoes remained same (Fig-26 C)
d. On 15th day of incubation it was observed that both negative control and positive control tomatoes were spoiled where there is no physical change in the treated tomatoes (Fig-26 D).

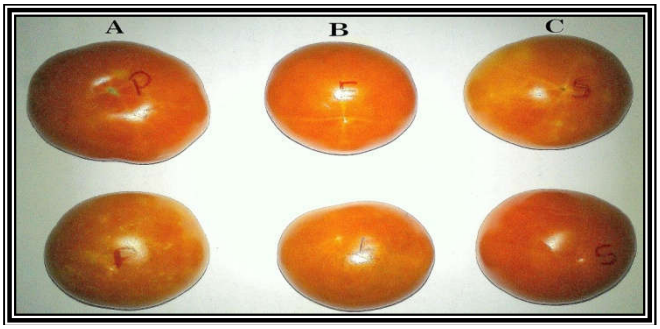


Fig 26 A Day 1 status
(Lane A = Negative control, Lane B = Treated, Lane C= Positive control)

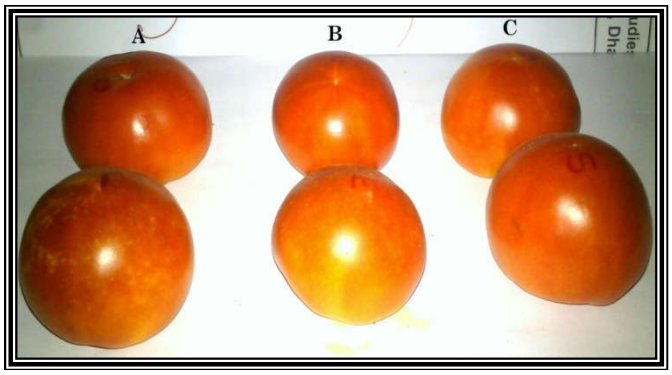


Fig 26 B Day 5 status
(Lane A = Negative control, Lane B = Treated, Lane C= Positive control)

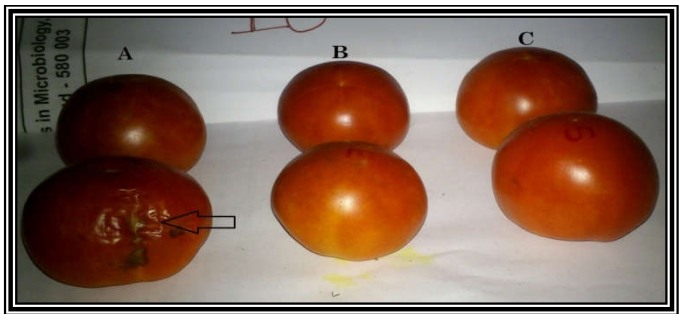


Fig 26 C Day 10 status
(Lane A = Negative control, Lane B = Treated, Lane C= Positive control)

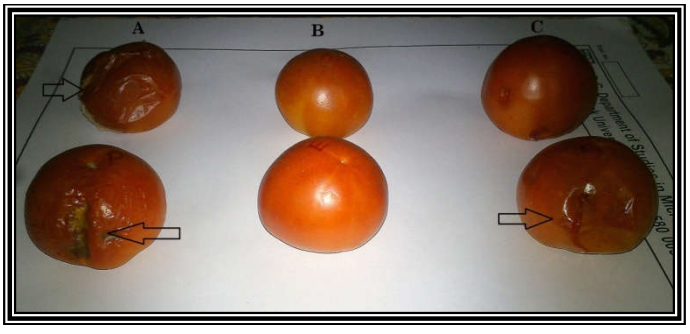


Fig 26 D Day 15 status
(Lane A = Negative control, Lane B = Treated, Lane C= Positive control)

The chemical constituents in the plants or crude extract are known to be biological active ingredient. Some chemical constituents are considered as secondary metabolites. They are directly responsible for different activity such as antioxidant, antimicrobial, and antifungal. *Tridax procumbens* has been

used as a phytomedicine by traditional healers and practitioners of Unani and Ayurvedic system of medicine (Mundada and Shivhare, 2010). In the present study it was evaluated that the different extracts of flower and leaves of *Tridax procumbens,* their phytochemical constituents, and efficacy of chloroform, ethanol and aqueous extracts of flower and leaves of the plant as antibacterial and antifungal agents against pathogens.

The therapeutic value of medicinal plant lies in the various chemical constituents present in it. A number of chemical constituents were reported from the plant *Tridax procumbens* VIZ : alkaloids, flavonoids, cerotenoids, lauric acid, palmtic acid, tannin (Singh *et al.*, 2004). Some experiments have revealed the presence of fumaric acid, saponin, carbohydrates and proteins. *Tridax procumbens* has been used as a phytomedicine by traditional healers and practitioners of Unani and Ayurvedic systems of medicine. This traditional usage stems from the fact that *Tridax* is associated with antibacterial activity (Mundada and Shivhare, 2010). We studied the efficacy of aqueous and ethanolic extracts of *Tridax* as antibacterial agents against human pathogens including nosocomial strains. While the ethanolic extract was associated with antibacterial activity the aqueous extract was not. Similar findings have been reported in another study (Aniel and Naidu, 2010). The difference in activity between the aqueous and alcoholic extracts can be explained by the fact that different solvents have varying capacities to extract phytoconstituents based on their solubility and polarity. The antibacterial activity of *Tridax* is known to be due to alkaloids, flavonoids, tannins and saponins (Mundada and Shivhare, 2010). Present study has shown that bioactive compounds are present in the extract of plant. In the preliminary phytochemical analysis, the three extracts of leaves (ethanol leaf extract, chloroform leaf extract, aqueous leaf extract) showed the presence of tannin , saponin, proteins , diterepenes, phenols, cardial glycosides, amino acids, coumarins and mostly alkaloids. Among these three extracts ethanol leaf extract showed good results. Flavonoids are known to be synthesized by plants in response to microbial infection, hence they have been found to be effective as antibacterial substance against a wide variety of infectious agents (Rajaram and Godghate, 2013). So in the present study it was found that flavonoids are strongly present in the ethanol flower and moderately present in the ethanol leaf, aqueous leaf and chloroform flower extracts. Earlier studies has shown that saponin fraction of *Tridax procumbens* has Immunomodulatory potential (Jindal *et al.,* 2013).

The GC-MS analysis of *Tridax procumbens* plant leaf extract showed the presence of important bioactive compounds such as α -pinene, β-pinene, l- phellandrene, Sabinene which found to have effect on antimicrobial and anti-inflammatory activity (Manjamalai *et al.,* 2012). The present study revealed that the probable compounds in the ethanolic flower extract was n-octocosane, Stearic acid, Myristic acid, Urcic acid, Oliec acid and n-Decyltetracosa. These compounds are used in softening agents in cosmetics, pharamaceutics, food industry as a preservatives.The crude extract of flower of *Tridax procumbens* have the antimicrobial activities and used against general food spoilage and human pathogens. Pharmacological studies have revealed that *Tridax procumbens* has antimicrobial activity against both gram positive and gram negative bacteria. Plant extracts inhibit the growth of microorganisms at different concentrations have also been reported (Salahdeen *et al.,* 2004). Some studies have evaluated that the ethanolic and ethyl acetate extract of *Tridax procumbens* possessed the greater inhibitory activity against both gram positive and gram negative bacteria (Malik *et al.,* 2012). Therefore the present study was conducted to assess the antimicrobial potential of chloroform, ethanol and aqueous extracts of flower and leaves of *Tridax procumbens.* In this study among the six extracts (ethanol leaf, chloroform leaf, aqueous leaf, ethanol flower, chloroform flower and aqueous flower), ethanol flower has shown the maximum activity against *staphylococcus aureus, Bacillus cereus and Salmonella typhi* representing its potential as a good antimicrobial agent.

In earlier studies it was revealed that minimum inhibitory concentration (MIC) of *Staphylococcus aureus* was sensitive to the ethanolic extract of *Tridax procumbens* (Aniel and Naidu, 2010). The ethanolic extract showed very good antibacterial activity only against gram negative non fermenters like *Pseudomonas aeruginosa*. There was no activity against gram positive as well as gram negative bacteria. These findings corroborate with some other studies (Mahato and Chaudhary, 2005). The *in vivo* antibacterial activity of *Tridax* against *Pseudomonas aeruginosa* in experimental animals has also been well established (Oladunmoye, 2006). In contrast to these findings, some other studies have demonstrated the efficacy of *Tridax* extracts even against other bacteria such as *S. aureus, E. coli, Klebsiella pneumoniae* and *Proteus* (Sharma and Kumar, 2009; Das *et al*., 2009; Yoga Latha *et al*., 2010). These differences could have arisen due to a number of factors.

In earlier studies antifungal activity of the methanolic extract of *Tridax procumbens* leaf was investigated in which the antifungal activity was assessed by zone of Inhibition using the minimal fungicidal concentrations of the extracts such as 150, 250, 500 µg/ml. The result reveals that the different concentration of the extract shows good antifungal activity when comparing with the positive control (Manjamalai *et al*., 2012). And in another study it was revealed that the chloroform, acetone and ethanol extract of *Tridax procumbens* have shown strong activity against *Penicillium* compared to *Aspergillus niger* (Priyadarshini *et al.,* 2013). The present antifungal activity of the six extracts of both leaves and flowers (chloroform, ethanol and aqueous) was studied by poison food technique. It was confirmed that at 5% concentration the growth of *Aspergillus niger* maximum inhibited by chloroform leaf, ethanol leaf and aqueous flower extracts. The growth of *Penicillium Sp* was inhibited maximum by aqueous leaf extract. The growth of *Rhizopus Sp.* maximum inhibited by chloroform flower and ethanol flower extracts. And at 10% concentration the growth of *Aspergillus niger* maximum inhibited by chloroform leaf, aqueous leaf, ethanol flower and aqueous flower. The growth of *Rhizopus Sp.* showed maximum inhibition by ethanol leaf and ethanol flower extract. These results indicates that the extracts of *Tridax procumbens* as an antifungal agent, however this is yet to be confirmed.

Enhancement of Shelf – Life of Tomatoes using herbal extracts was tried earlier (Surekha *et al.,* 2010). The present study was therefore conducted to check the efficiency of the extracts to increase the shelf life of tomatoes and our results confirms that the ethanol flower extract of *Tridax procumbens* has extended shelf life of tomatoes when ethanol flower extract is coated on it.

CONCLUSION

The present study revealed that the plant *Tridax procumbens* acts as a potential source of useful drug. The phytochemical screening has shown the presence of flavonoid, tannin, saponin, protein, alkaloids, coumarin etc. The antimicrobial activity suggests that they can be used for treatment of the diseases as traditional healers. Ethanolic extract of *Tridax Procumbens* exhibited remarkable antibacterial activity against pathogenic bacteria particularly against *Staphylococcus aureus, Bacillus cereus, Salmonella typhi,* and antifungal activity against fungi like *Aspergillus niger, Penicilium Sp. and Rhizopus Sp.* Hence*, Tridax procumben*s can act as a source of new antibiotic compound for preparing herbal drug for the treatment of diseases caused by these pathogens.

Our results also confirmed that ethanolic flower extract of *Tridax procumbens* has extended the shelf life of tomatoes. Hence, it can be exploited as a herbal food preservative. The present study shows the presence of phytochemical in the *Tridax procumbens* which might be the reason for these inhibitions and essentially contain herbal bioactive compounds like Oleic acid, stearic acid and erucic acid which require further structural elucidation and characterization methodologies to identify the bioactive constituents. The present study reveals that *Tridax procumbens* can be considered as promising source of an antibacterial and antifungal activity, it can also be used as herbal food preservative. In future, there is huge room for research in direction of more pharmacological activities of *Tridax procumbens* and to elucidate the mechanism of action of same.

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