

## Antibacterial, antioxidant, wound healing activities and stop-bleeding effect of methanolic extract of *Tridax procumbens* L. leaves

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### Abstract:

*Tridax procumbens* L. is a leafy herb commonly used in different traditional treatments in many countries including Yemen. The main aim of the present study is evaluating some specific biological activities (antibacterial, antiradical, wound healing potential and stop-bleeding effect) of methanolic (MeOH) extract of *T. procumbens* leaves collected from Yemen. Antibacterial effect was examined by paper disc diffusion method against different bacterial species, antioxidant was evaluated in vitro against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals, wound healing and stop-bleeding were evaluated in experimental animals. Phytochemicals were screened by qualitative standard methods. Results showed that, MeOH extract of *T. procumbens* exhibited a strong anti-DPPH radical effect with IC<sub>50</sub> value 151.23 µg/mL. Antibacterial activity was only observed against *Klebsiella pneumoniae* with the highest inhibition zone 16 mm by 100 mg/mL of extract. Treatment applications with different concentrations used of MeOH extract showed completely healing for wounds on the 11<sup>th</sup> day of treatment and reduced time of bleeding by 50% compare to control (P<0.05). Major secondary phytochemicals were detected in MeOH extract of *T. procumbens*. The obtained results in this study indicated that, *T. procumbens* could be used as a natural source for many forms of pharmacological preparations. Further clinical and toxicological studies are recommended.

**Keywords:** Biological activities, Phytochemicals, *Tridax procumbens*, methanolic extract.

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## Introduction:

Medicinal herbs represent an effective agent and accessible source has been used for decades in therapeutic applications around world especially in developing countries. When the population in rural regions depend mainly on natural resources as herbal medicine in their treatment of many ailments as chronic inflammation and severe infection (Tabuti et al.2003; Ved and Goraya, 2007). *Tridax Procumbens* (Asteraceae) is a medicinal herb has been used for long time in traditional medicine in Africa, South and Southeast Asia with wide treatment applications as wound healing, haemostatic, antiseptic and pain relieving (Dattaray 2022), and to cure of bronchial catarrh, diarrhea, dysentery and liver diseases (Andriana et al.2019). Recently many in vivo and in vitro studies were carried out on *T. procumbens* which proved a wide range of pharmacological activities as antimicrobial and antiparasite (Naqash and Nazeer, 2011; Policegoudra et al.2014; Gamboa-leon et al.2014; Kumar et al.2016), antioxidant (Habla et al.2010; Singh et al.2017; Syed et al.2020), anti-inflammatory and immunomodulatory (Jachak et al.2011; Tiwari et al.2004), and anticancer (Syed et al.2020;) activities (Table 1 show the main pharmacological properties of *Tridax Procumbens* reported in recent studies). The mentioned biological activities of *T. procumbens* are always attributed to its content of bioactive phytochemicals as phenols (thymol, eugenol, Gallic acid and isobutyl gallate) (Dattaray, 2022); fatty acids (palmatic acid, arachidic acid and lauric acid) sterols (b-amyron, b-amyrin, stigma sterol, lupeol, luteolin, campasterol), alkaloids and flavonoids molecules (Surendra et al. 2016; Surendra et al.2016; Gurusamy et al.2019; Dattaray 2022). In developing countries wounds are still a major problem, often having severe complications due to the delay of treatment in the most cases with the high costs for therapy by chemical drugs (Sharma et al.2021). Also, with the delay of treatment, the wounds may be infected with many pathogens as bacteria. Therefore, there is an urgent need to search for inexpensive sources and safe alternative agents to solve these problems. Several medicinal plants represent an accessible and natural source for treatment many diseases including injuries and bacterial infections. In Yemen *T. procumbens* is widespread and well growing between the rocks, along roadsides and in the agronomic fields with the annual crops. It is traditionally applied for curing wounds by squeezing its fresh leaves directly on the fresh injuries to stop bleeding and sometimes its fresh leaves are prepared as paste and applied on the wound. Previous studies reported a potent wound healing effect of *T.*

*procumbens* from different countries (Yaduvanshi 2011; Udopa et al.2014; Ambulkar et al.2020; Kathiresan et al.2021). And few reports examined its effect on the coagulation and time of bleeding (Ikese et al.2015; Gubbiveeranna et al.2019). In Yemen no report was conducted on the pharmacological properties of *T. procumbens* especially those properties related to its locally traditional medication as wound healing, antiseptic and anti-bleeding activities. Therefore, the current study aims to evaluate the effect of methanolic extract of *T. procumbens* leaves on the cutaneous wounds and on the time of bleeding in the experimental animals as well to determine the antibacterial and antiradical activities in vitro.

Table 1. Pharmacological properties of *Tridax procumbens*

Plant Part	Extract	Biological activity	Phytochemical	Reference
Whole plant	Methanol and aqueous	Antioxidant and antibacterial activities	Phenolic and flavonoids compounds	Bera and Banerjee (2023)
Leaves	Dichloromethane, butanol, ethyl acetate and methanol	Antidiabetic and antioxidant activities	Flavonoids, phenols, terpenes, sterols and alkaloids	Nandi et al. (2022)
Whole plant	Hydroalcoholic extract and fractions	Antioxidant and antimicrobial activities	Flavonoids, phenols, terpenes, sterols and alkaloids	Jalalpure and Patil (2022)
Leaves	Methanol, ethanol, aqueous, chloroform, acetone and ethyl acetate	Anticancer, Antioxidant and antibacterial activities	Alkaloids, polyphenols and tannins	Syed et al. (2020)
Different parts (leaf, stem and flowers)	Ethanol 96%	Antibacterial activity	Flavonoids, terpenoids, tannin, alkaloids and saponin	Arma et al. (2022)
Different parts (leaves, stem and flowers)	Hexane and methanol	Antioxidant activity	Terpenes and sterols	Mahdi (2023)
Whole plant	Aqueous	Wound-healing and antibleeding activities	Phenols	Gubbiveeranna et al.(2019)

## Materials and Methods:

### Plant materials

The aerial part (leaves, stem and flowers) of *T. procumbens* were collected from Almahweet province, Yemen and plant was authenticated at Department of Biology, Hajjah University, Republic of Yemen.

### Extraction process

Fresh leaves of *T. procumbens* were cleaned by washing in tap water for five minutes then, dried at room temperature for one week, after that the dried leaves were ground to fine powder by mortar and pestle and 50 g of powder was macerated in 500 mL methanol at room temperature for 3 days, then the extract was filtered by filter paper (Whatman No.1) and filtrate was evaporated under vacuum to get a dried solid product and stored in dried bottles at 4 °C.

### Phytochemical examination

Phytochemical screening for bioactive secondary metabolites in studied plant were carried out using different standard methods according to that described by Harborne (1992), with some modifications were done. Briefly the following treatments were conducted:

About 5 g of plant material was dissolved in 50 mL distilled water as stock solution, then 0.5 mL of prepared stock solution was taken and treated with:

- 1- One mL of dilute hydrochloric acid and a few drops of Dragendorff's reagent for detecting the presence of alkaloid as a prominent yellow precipitate in mixture.
- 2- Drops of neutral 5% ferric chloride for detecting the presence of phenolic components as a dark green colour.
- 3- Drops of sodium hydroxide solution after adding drops of dilute HCl for detecting the presence of flavonoid as intense yellow colour.
- 4- About 2 mL of chloroform and drops of sulfuric acid for detecting the presence of steroids and terpenoids as a reddish brown colour ring.

### Antibacterial assay

Antibacterial test was implemented according to paper disc diffusion method described by Barberis et al.(2018) with minor modifications. Five bacteria strains [(*Bacillus subtilis* ( $G^{+Ve}$ ), *Staphylococcus aureus* ( $G^{+v}$ ), *Escherichia coli* ( $G^{-V}$ ), *Pseudomonas aeruginosa* and

*Klebsiella pneumoniae*( $G^V$ ) were grown in molten nutrient agar medium by incubation at 37 °C for 24 hours. The final population of all strains was standardized to be about  $10^8$ -  $10^9$  CFU/ mL. A volume of 0.02 mL of bacteria culture was covered evenly on the surface of dried agar plates (diameter = 9 cm). Afterward, a volume of 0.02 mL of plant extract was dissolved in %10 DMSO, then the dissolved extract was applied into filter paper discs (6 mm diameter) and placed on the surface of the tested bacteria plates. After 24 h of incubation at 37 °C, the inhibition zones were measured. Reference drug (Ciprofloxacin 5 µg /disc) and %10 DMSO were used as the positive and negative controls respectively.

### Wound healing assay

Wound healing experiment was implemented on male rabbits (n=15 animal; weight 450 to 500 g). Rabbits were randomly divided into five groups each group contain three animals [group negative control (without treatment); positive control group (treated with povidone iodine); experimental groups (treated with 12.5 mg/mL; 25mg/mL and 50 mg/mL of plant extract). The dorsal skin of each animal in all groups was shaved and cleaned with betadine under anesthesia, and one open full-thickness wound that approximately  $1.0 \times 1.0$  cm was incised up to the level of subcutaneous adipose tissue by means of a surgical blade. After 24 h following the wounds in the control and experimental groups were treated topically daily. Area of wounds was measured every day, from the first day to 12th day. Data were analyzed via SPSS software (ver.16) at the significant level of  $P < 0.05$ .

### Bleeding time assay

Effect of plant extract on bleeding time was determined according to the previous method of Ikese et al.(2015) with some modifications. Experiment was conducted on male rabbits; five groups were used same to that described in wound healing assay with only positive control here was vitamin K. The ear flap of each animal was shaved and sterilized with cotton dabbed in ethanol (70%). Then, using a sterile lancet about 3 mm deep laceration was done on the ear flap of rabbit and after removing the first drop of bleeding a drop of different treatments; plant extract (50mg/mL,25mg/mL and 12.5mg/mL), vitamin K and control (no treatment) were applied directly onto the site of laceration. The time of experiment for all treatments was calculated using a stop watch from the time of applied treatment till stop of

bleeding, also the drops of bleeding were amounted. The means of bleeding time were calculated and compared using t-test.

### Anti-DPPH Radical assay

*In vitro* anti-radical scavenging activity was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) modified method as described previously by Elzaawely and Tawata (2012). Graded levels of plant extract 50,100,150,200,250 and 300 µg/mL were prepared by dissolving in methanol. Final volume of test mixture consisted of 100µL of sample and 100µL of 0.2 mM DPPH solution.

The mixture was put in 96-well microtiter plates, kept in dark for 30 min at room temperature and the optical density was then measured at 517 nm using Cecil-Elect. Spectrophotometer. Butylated hydroxytoluene (BHT) was used as reference antioxidant compound. Percentage anti-DPPH scavenging activity was amounted according to the following equation:

$$\text{Percent (\%)} \text{ inhibition of DPPH activity} = \frac{(\text{Abs. Sample} - \text{Abs. Control})}{\text{Abs. Control}}$$

The IC<sub>50</sub> values were calculated.

### Statistical analysis

Experiment data were statistically analyzed using SPSS version19 (Chicago, IL, USA). The values were expressed as mean ± standard deviation (SD). Significant differences between samples were detected by analysis of variance (ANOVA) followed by Duncan's multiple-range test ( $p < 0.05$ ).

## Results and Discussion:

### Phytochemical screening

Screening the presence of different phytochemicals in MeOH extract of *T. procumbens* showed that the extract was rich in major bioactive secondary metabolites as alkaloids, tannins, phenols, flavonoids, steroids and terpenoids, results are illustrated in table 2.

Table 2. Screening secondary metabolites in MeOH extract of *T. procumbens* leaves.

Test	Reagent	Result	Observation
Alkaloids	Dragendorff's	++	yellow precipitate
Phenolic compounds	FeCl <sub>3</sub> (5%)	+++	bluish black color
Tannins	FeCl <sub>3</sub> (1%)	++	bluish black color
Steroids and Terpenoids	Salkowski's test	+++	reddish brown color ring
Flavonoid	NaOH (10 %)	++	yellow color

Key: (+) Positive Test; (-) Negative test; (+) weak; (++) moderate; (+++) strong

These results are in the line and confirm that reported in many reports, which proved the presence of the mentioned phytochemicals in different extracts from different parts of *T. procumbens* as MeOH of leaves (Dhanabalan,2008); ethanol of whole plant (Petchi et al.2013) and aqueous extract of leaves (Singh et al.2017). Nandi et al.(2022) revealed the presence of flavonoids, phenols, terpenes, sterols and alkaloids in different extracts including MeOH of *T. procumbens* and they were the main responsible of antidiabetic and antioxidant and activities. Also, Syed et al.(2020) reported anticancer, antioxidant and antibacterial activities of *T. procumbens* leaves extracts and the detected activities were imputed to the rich of *T. procumbens* leaves extracts in alkaloids, phenols and tannins. Furthermore, different parts (leaves, stem and flowers) of *T. procumbens* were extracted by hexane and MeOH revealed the presence of terpenes and sterols with a strong antioxidant activity (Mahdi 2023).

*T. procumbens* phytochemicals are the main contributors of its pharmacological properties and therapeutic benefits. Flavonoids and tannins of *T. procumbens* with their antioxidant activity were also able to exert a strong antibacterial activity by several mechanisms against microorganisms as inhibition cytoplasmic membrane function, inhibition DNA gyrase, and inhibition metabolism energy (Pai et al.2011). And steroids and terpenoids are acted as antibacterial and anti-inflammatory agents (Manjamalai et al.2012; Andriana et al.2019). While alkaloids and tannins were described as stimulators for fast healing and contracting of cutaneous wounds (Tsala et al.2013; Begashaw et al.2017).

Table 3. Antibacterial activity of MeOH extract of *T. procumbens* against standard microorganisms.

Extracts	Mean of inhibition zone (mm)+SD**				
	G <sup>+Ve</sup>		G <sup>-Ve</sup>		
Cons. mg/mL	<i>B.s</i>	<i>S.a</i>	<i>E.c</i>	<i>K.p</i>	<i>Ps.a</i>
20mg/mL	-	-	-	12±0.0 <sup>b</sup>	-
50mg/mL	-	-	-	15.33±0.0 <sup>a</sup>	-
100mg/mL	-	-	-	16±1.0 <sup>a</sup>	-

### Antibacterial activity

Table3 shows the antibacterial activity of plant extract by measuring the inhibition zone diameter (mm) against different standard tested microorganisms.

\*\*SD= standard deviation.

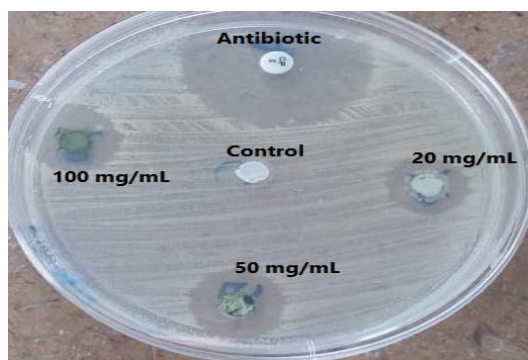
*B.s*= *Bacillus subtilis*, *S. a*= *Staphylococcus aureus*, *E. c*= *Escherichia coli*,

*Ps. a* =*Pseudomonas aeruginosa*, *K.p* = *Klebsiella pneumoniae*

(-) = Not effective

Different superscript letters indicate a significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.

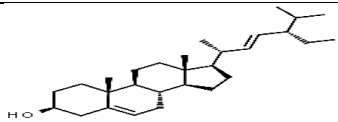
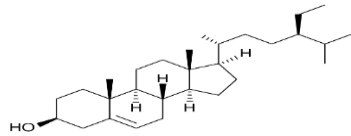
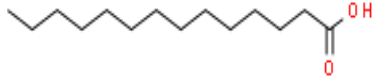
Three concentrations (20,50 and 100 mg/mL) of extract were prepared to detect their effect against studied microorganisms. Aantibacterial activity of plant extract was only observed against *K. pneumoniae* with inhibition zone 12 mm,15.33 mm and16 mm by 20,50 and 100 mg/mL of extract respectively (Figure 1).

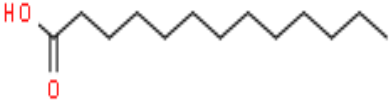
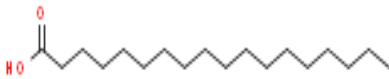
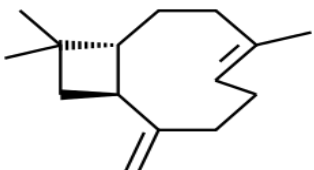
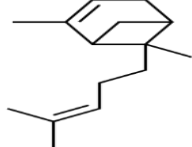
Figure 1. Inhibition zone by MeOH extract of *T. procumbens* against *K. pneumoniae*.

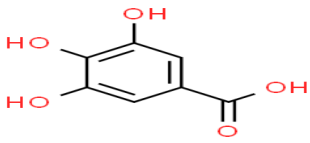
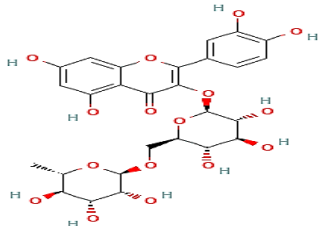
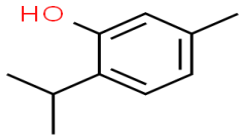
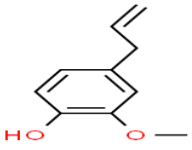
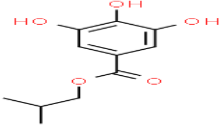
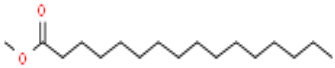
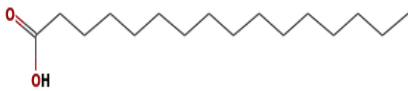


Other examined bacteria were resistance to plant extract at all concentrations used. Different antibacterial effects of *T. procumbens* were early reported against varied bacterial species and the effect was depending on the type of solvent used in extraction. Bhati-Kushwaha and Malik (2014) and Saritha et al.(2015) reported antibacterial activity of aqueous, ethanol, and MeOH extracts of *T. procumbens* only against *E. coli*, while the ethyl acetate extract had a wide range of antibacterial activity against *E. coli*, *B. cereus*, *K. pneumoniae*, *S. aureus*, *S. typhi* (Bharati et al.2012). Also, Andriana et al.(2019) found a potent antibacterial activity of *T. procumbens* ethyl acetate fractions against different gram positive and gram negative strains, and their effect was ascribed to the presence of stigmasterol,  $\beta$ -sitosterol, and n-hexadecanoic acid (Table 4 show more details about component; chemical structure, formula and biological activities) in the active fractions. Furthermore, Syed et al.(2020) identified six compounds; n-Hexadecanoic acid, Tetradecanoic acid, Tridecanoic acid, Octadecanoic acid, Caryophyllene, trans- $\alpha$ -Bergamotene (Table 4) using GC/MS analysis of MeOH extract of *T. procumbens* leaves and the detected compounds have various therapeutic potentials including antimicrobial activity (Ramesh et al.2013).

Table 4. Bioactive components detected in different parts of *Tridax procumbens*

Compound name	Chemical structure	Formula	Biological activity (Reference)
Stigmasterol		$C_{29}H_{48}O$	Anti-inflammatory (Gomez et al. 1999; Akihisa et al.2000) and antibacterial (Andriana et al.2019) activities
$\beta$ -Sitosterol		$C_{29}H_{50}O$	Anti-inflammatory (Gomez et al. 1999) and antibacterial (Andriana et al. 2019) activities
Tetradecanoic acid		$C_{14}H_{28}O_2$	Antibacterial (Andriana et al.2019) activity and anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activities (Ramesh et al.2013).

Tridecanoic acid		$C_{13}H_{26}O_2$	Antibacterial (Andriana et al.2019) activity and anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activities (Ramesh et al.2013).
Octadecanoic acid		$C_{18}H_{36}O_2$	Anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activities (Ramesh et al.2013).
Caryophyllene		$C_{15}H_{24}$	Anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activities (Ramesh et al.2013).
trans- $\alpha$ -Bergamotene		$C_{15}H_{24}$	Anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activities (Ramesh et al.2013).

Compound name	Chemical structure	Formula	Biological activity (Reference)
Gallic acid		$C_7H_6O_5$	Antioxidant, antimicrobial and anti-inflammatory activities (Ghasemzadeh and Ghasemzadeh, 2011), anticoagulant activity (Amirou et al.2022).
Rutin		$C_{27}H_{30}O_{16}$	Anticoagulant (Amirou et al.2022); antioxidant and anticancer activities (Ali et al.2008, Ghasemzadeh et al. 2010).
Thymol		$C_{10}H_{14}O$	Antioxidant activity (Singh et al.2017), antioxidant and anti-inflammatory activities (Dattaray, 2022).
Eugenol		$C_{10}H_{12}O_2$	Antioxidant activity (Singh et al.2017), anti-inflammatory and immunomodulatory (Dattaray, 2022).
Isobutyl gallate		$C_{11}H_{14}O_5$	Antioxidant activity (Singh et al.2017), anti-inflammatory (Dattaray, 2022).
Methyl palmitate		$C_{17}H_{34}O_2$	Antioxidant activity (Andriana et al. 2019)
n-Hexadecanoic acid		$C_{16}H_{32}O_2$	Antibacterial activity (Andriana et al. 2019; Syed et al.(2020) and anticancer, immune suppressive, anti-spermatogenic, antineoplastic, antiarthritic, anti-inflammatory and antimicrobial activities (Ramesh et al.2013).

### Wound healing activity

Results in the figure 2 revealed that, the area of wounds reached to be zero on 11<sup>th</sup> day of treatment in all concentrations used (50 mg/mL, 25mg/mL and 12.5mg/mL) of extract, and

insignificant difference ( $P>0.05$ ) was observed between them, that means an approximately equal effect and completely healing for wounds treated with these concentrations employed of extract after 11<sup>th</sup> day of treatment. The wounds of rabbits treated with standard drug (povidone iodine) and control were not completely healed in the most rabbits till after 11<sup>th</sup> day of treatment compared to the wounds treated with plant extracts which were better and faster healed ( $P<0.05$ ).

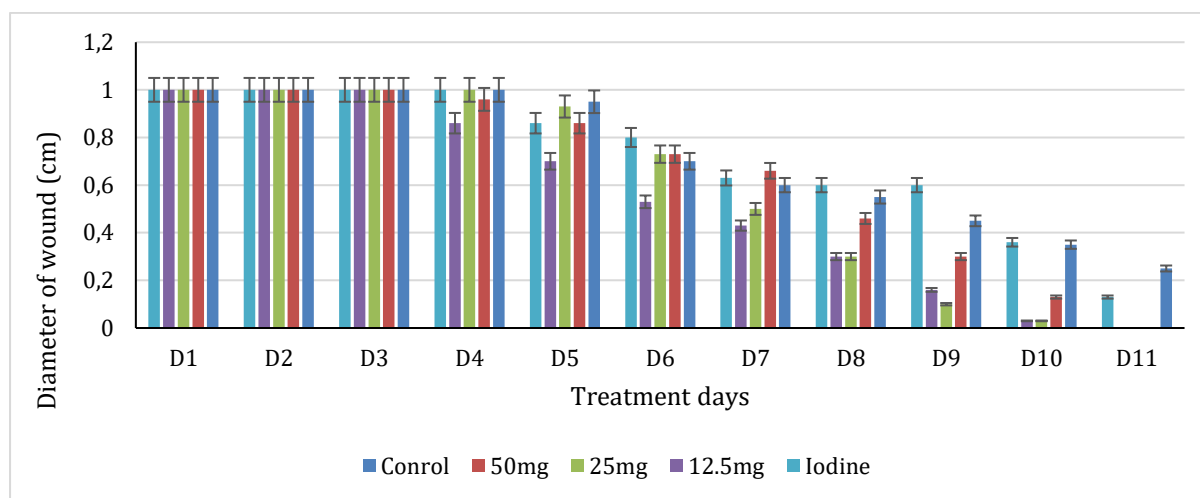


Figure 2. Effect of different concentrations of MeOH extract of *T. procumbens* leaves on wound healing during 11 day of treatment.

These findings are in agreement with that previously reported by Ambulkar et al. (2020) when they recorded a significant and potent effect of different dosages used of *T. procumbens* in curing and contracting cutaneous wounds in experimental rats compare to control groups. Also, Dattaray (2022) results showed that, the ethanolic extract of *T. procumbens* was quite more effective in increasing wound contraction compared to the effect of aqueous extract. And this higher effect was due to rapid collagen formation by alcoholic extract (ethanol) of *T. procumbens* compared to the effect of aqueous extract. These effects are frequently imputed to *T. procumbens* phytochemical contents, as alkaloids which act as stimulators for colonies growth from fibroblast precursors in the early phase of healing (Begashaw et al.2017), and tannins which act as astringents factors and phenolic components which play as strong anti-inflammatory and antioxidant agents (Tsala et al.2013).

### Effect of *T. procumbens* MeOH extract on the bleeding

Table 5 displays the results of the time of stop bleeding (measured by seconds) and the drops of blood were also amounted in all treatments; different concentrations of plant extract (50 mg/mL, 25mg/mL and 12.5mg/mL), control (no treatment) and drug (vitamin K). The mean of the time of end bleeding in control was  $42.5 \pm 3.53$  Sec. with  $4.5 \pm 0.7$  drops of blood were amounted. The treatment applications with plant extract (50 mg/mL, 25mg/mL and 12.5mg/mL) significantly ( $P < 0.05$ ) dropped the time of bleeding by approximately 50% to be  $20 \pm 0.0$  Sec.,  $22.5 \pm 0.7$  Sec. and  $19.5 \pm 0.7$  Sec. respectively compared to control, with 2.5 drops of blood were calculated for all concentrations used.

Table 5. Effect of *T. procumbens* MeOH extract on the bleeding

Different superscript letters indicate a significant difference ( $p < 0.05$ ) according to Duncan's multiple range test.

The results of plant extract could be comparable to that obtained by standard drug when the time of end bleeding was  $11 \pm 1.4$  Sec. *T. procumbens* plant especially its leaves are

Treatment		Time of stop bleeding (Sec.)	No. of Blood Drops
<b>Negative control</b>	No treatment	$42.5 \pm 3.53^a$	$4.5 \pm 0.9^a$
	50mg	$20 \pm 0.0^b$	$2.5 \pm 0.7^b$
<b>Plant extract</b>	25mg	$22.5 \pm 0.7^b$	$2.5 \pm 0.6^b$
	12.5mg	$19.5 \pm 0.7^b$	$2.5 \pm 0.1^b$
<b>Positive control</b>	Vitamin K	$11 \pm 1.4^c$	$1.0 \pm 0.0^c$

traditionally applied on fresh wounds to stop bleeding and to improve the wound curing process (Gubbiveeranna and Nagaraju 2016). To prove that, some scientific reports were conducted, as that carried out by Kale et al. (2008) when their results reported a potent haemostatic effect of ethanolic extract of *T. procumbens* by reducing the clotting time in the all examined blood samples. Also, Ikese et al.(2015) confirmed that effect, when the aqueous extract of *T. procumbens* leaves decreased the bleeding time by 57%. Furthermore, Gubbiveeranna et al.(2019) study recorded potent procoagulant and platelet aggregation effects of *T. procumbens* leaves aqueous extract and the obtained effect was explained by inducing serine protease enzyme. Yahia et al.(2023) imputed hemostatic activity of

methanolic extract of *Atriplex halimus* leaves to the phenolic compounds when they found a significant linear correlation between the values of phenolic compounds and hemostatic activity, the phenolic compounds were the main contributors of anticoagulant activity. Also, flavonoids isolated from *Juglans regia* as gallic acid and rutin components (Table 4) recorded a potent antithrombotic effect via antiaggregant and anticoagulant actions. Their effect was implemented by inhibition platelet aggregation and by prolong plasmatic coagulation times (Amirou et al.2022).

### Antioxidant activity

Results of anti-DPPH free radical scavenging activity of tested extract are presented in table 6.

Table 6. Anti-DPPH radical scavenging activity of MeOH extract of *T. procumbens*.

Conc. $\mu\text{g/mL}$	DPPH Scavenging activity (%)	*IC <sub>50</sub> ( $\mu\text{g/mL}$ )
50	6.43±0.51 <sup>f</sup>	
100	32.33±2.51 <sup>e</sup>	
150	46.33±1.51 <sup>d</sup>	
200	60±2.0 <sup>c</sup>	151.23±0.00 <sup>a</sup>
250	68.66±1.15 <sup>c</sup>	
300	83.66±3.21 <sup>b</sup>	
BHT**	92.8±1.86 <sup>a</sup>	64.90 ± 0.75 <sup>b</sup>

Different superscript letters in the same column indicate significant difference ( $p < 0.05$ ).

\*Inhibition concentration 50 (IC<sub>50</sub>). \*\* The concentrations used of BHT were exactly the same as that used of plant extract.

DPPH radical scavenging activity of the tested levels of extract were concentration dependent. Maximum anti-DPPH radical effect 83.66±3.21% was observed in the highest concentration (300  $\mu\text{g/mL}$ ) of plant extract. Significant differences ( $p < 0.05$ ) were detected among antioxidant potential of the different concentrations used of plant extract.

The IC<sub>50</sub> values were also calculated for plant extract and compared with that of BHT. MeOH extract of *T. procumbens* showed IC<sub>50</sub> value 151.23  $\mu\text{g/mL}$  which was comparable to that of standard BHT (64.90 ± 0.75  $\mu\text{g/mL}$ ). These findings support many previous results which proved antioxidant potential of *T. procumbens* by different in vitro and in vivo

methods, as that displayed by Jachak et al. (2011) study which showed higher antioxidant activity by methanolic extract against DPPH and ABTS radicals with IC<sub>50</sub> value 45.95 µg/mL and 24.47 µg/mL respectively. Also, Syed et al.(2020) reported less anti-DPPH inhibition percent (52.16 ± 0.21%) by MeOH extract compare to our results. This variation in the antioxidant potential may be due to the varying of the content of phenol and flavonoid in plant which are the major responsible of antioxidant activity. Singh et al.(2017) results revealed the presence of four phenolic compounds namely thymol, eugenol, gallic acid and isobutyl gallate (Table 4) in MeOH extract of *T. procumbens* leaves using GC/MS analysis and the detected components were the main responsible of antioxidant activity. Furthermore; Andriana et al.(2019) study, showed the strongest anti-DPPH and anti-ABTS radical activities were evaluated for some ethyl acetate extract fractions of *T. procumbens* and their effect was attributed to some dominant compounds detected by GC/MS analysis as methyl palmitate (Table 4). Antioxidant activity is frequently imputed to the phenolic contents in many plants, Rahhal et al.(2020) reported that, when they found a positive correlation was detected between phenols, flavonoïds and antioxidant activity in methanolic extracts of 14 *Juniperus thurifera* L. stands collected from the High and the Middle Atlas Mountains of Morocco.

## Conclusion:

*T. procumbens* is an accessible and a famous herb with wide therapeutic applications, predominantly used in arresting bleeding and curing cutaneous wounds in many countries including Yemen. The current work for the first time evaluated some biological activities of *T. procumbens* which are related to its traditional treatments in Yemen in order to prove that and to compare its medicinal benefits with that published abroad. MeOH extract of *T. procumbens* leaves had a considerable antioxidant activity against DPPH radical in vitro with IC<sub>50</sub> value 151.23±0.00 µg/mL, and adequate antibacterial effect was measured against *K. pneumoniae* as well as an effective wound healing and stop-bleeding potentials were evaluated in experimental animals. These activities confirm its traditional applications and based on that we could recommend it as natural material for many pharmacological and cosmetically preparations. The future studies should be focused on the isolation and determination of bioactive molecule(s) responsible for the biological activities of *T.*

*procumbens* and their mode of action are warranted.

### Acknowledgment:

The present study was implemented at Modern Specialized University, Sana'a, Yemen. The authors are thankful to president of Modern Specialized University Dr. Mojahid Aljober for providing laboratory facilities.

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*Arabian Journal of Medicinal and Aromatic Plants,*  
*ISSN 2458-5920, [www.ajmap.info](http://www.ajmap.info)*