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Original Article

# Antioxidant potential of chitosan-*Tridax procumbens* ethanol leaf extract composite

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## ABSTRACT

The increasing concern over oxidative stress-related diseases and the limitations of synthetic antioxidants have driven the search for natural, safe, and effective alternatives. This study aims to develop a chitosan–*Tridax procumbens* extract composite and evaluate its antioxidant potential using standard in vitro assays. Ethanolic extracts of *T. procumbens* leaves were incorporated into chitosan to form a bioactive composite film via solvent casting. The antioxidant activity of the composite was assessed using DPPH radical scavenging and Ferric Reducing Antioxidant Power (FRAP) assays. The result revealed that *T. procumbens* leaves had several phytochemicals, however, phenol was significantly ($p<0.05$) higher in concentration (439.96 ± 0.68 mg/100g) whereas tannins had the lowest in concentration (9.36 ± 0.35 mg/100g) compared to other phytoconstituents. Chitosan had significantly ($p<0.05$) lower DPPH and FRAP scavenging ability (29.87±0.32 to 57.05±0.55 %) and (38.46±1.08 to 79.87±0.76 %) respectively at all the concentrations (50-500 µg/ml) compared with those of the free plant extract and the standard ascorbic acid. The composite demonstrated significantly enhanced antioxidant activity compared to chitosan and *Tridax procumbens* extract alone, with DPPH scavenging and FRAP values significantly ($p<0.05$) exceeding those of the free extract and chitosan alone. The synergistic effect is attributed to the interaction between chitosan's functional groups and the phenolic constituents of *T. procumbens*, which may enhance stability and radical neutralization efficiency. The chitosan–*Tridax procumbens* composite exhibits potent antioxidant activity and holds promise as a multifunctional biomaterial for various applications.

**Keywords:** *Tridax procumbens*, chitosan, antioxidant activity, DPPH, FRAP

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## INTRODUNCTION

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defenses, is implicated in numerous chronic diseases and accelerated aging. There is

a growing interest in natural antioxidant materials to mitigate these effects, particularly within biomedical and food packaging applications [1].

*Tridax procumbens*, a medicinal plant from the Asteraceae family, has gained attention in recent years for its wide-ranging bioactivities, including antioxidant, anti-inflammatory, and wound-healing effects. In 2024, Nandita and colleagues formulated a chitosan gel enriched with *T. procumbens* leaf extract, demonstrating both DPPH and hydroxyl radical scavenging activities comparable to commercial antioxidants [2]. Additionally, a nanohydrogel combining chitosan nanoparticles and *T. procumbens* extract significantly boosted cell viability and migration in human gingival fibroblasts—evidence of synergistic therapeutic potential [3].

Chitosan, derived from chitin, is renowned for its biocompatibility, biodegradability, and mild antioxidant properties, which stem from its amino and hydroxyl functional groups. A 2022 meta-analysis highlighted chitosan's ability to enhance antioxidant enzyme activities and plant defense responses by approximately 40 %, underscoring its intrinsic redox-reducing capacity [4].

Incorporating plant-derived antioxidants into chitosan matrices has emerged as an effective strategy to enhance stability, control release, and amplify activity. For example, chitosan films embedded with Ficus extract exhibited significantly enhanced antioxidant properties [5]. Similarly, chitosan blends with other phenolic-rich plant extracts have exhibited improved DPPH and FRAP activity, physical strength, and UV protection—features desirable for active packaging [6,7].

However, despite these advances, the specific potential of chitosan–*T. procumbens* composites remain underexplored. There is dearth of information on the antioxidant potentials of chitosan-*Tridax procumbens* extract composite. Considering the rich phytoconstituents in *T. procumbens* (especially phenolic content) as well as the synergistic scaffold provided by chitosan, such composites could yield materials with enhanced antioxidant function and versatile application such as in pharmaceuticals, food preservation, and wound care amongst others. Therefore, the present study aims to evaluate the antioxidant potential of chitosan–*Tridax procumbens* composite using in vitro assays such as DPPH and FRAP. This study seeks to bridge the gap in current literature and establish a foundation for functional biocomposite applications.

## MATERIALS AND METHODS

### Sample Collection and Preparation of Plant Extract

The chitosan was obtained from Wisapple Biotech. Co., Ltd, Beijing and the plants (*Tridax procumbens*) was collected from Oziokutu community, Kogi State, Nigeria. The leaf and stem were separated, washed thoroughly with tap water and were transported to the Department of Plant Biology, Federal University of Technology Minna, Niger State for identification. After identification the sample was shade dried for 10 days and pulverized using a mixer grinder. The leaf powder was used for the extraction of the phytoconstituents.

### Extraction Process

Extraction of plant sample was done by cold maceration with 95 % ethanol as reported by Nerleker *et al.* [8]. Exactly 100 g of the sample was weighed into a 1000 ml beaker and 250 ml of 95 % ethanol was added into it. The solution was left to stand for 3 days for thorough

and effective extraction, and then the solution was filtered using clean muslin cloth. The liquid portion was collected and left to dry by evaporation at room temperature, the crude extract was collected and weighed, and the percentage yield was calculated.

### Quantitative Determination of the Phytochemicals

#### Determination of total flavanoids

Aluminium chloride colorimetric method was used for flavonoids determination as reported by Chang *et al*. [9]. The plant extract (0.5 g) was mixed with 1.5 ml of methanol, 0.1 ml of 10 % aluminium chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of distilled water. It was allowed to stand at room temperature for 30 min; the absorbance of the mixture was measure at 415nm with a double beam spectrometer. The calibration curve was prepared by preparing quercetin solution.

#### Determination of total phenol content

The total phenol content of the extract was determined using the method reported by Ji-Hye *et al*. [10]. Appropriate dilutions of the extract (0.5 ml) were oxidized with 2.5 ml of 10 % Folin-Ciocalteau's reagent (v/v) and neutralized by 20 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 $^{o}$C and the absorbance was measured at 75 nm in the spectrophotometer. The total phenol content was subsequently calculated using gallic acid as standard.

#### Determination of alkaloids

Half gram (0.5 g) of the sample was dissolve in 90 % ethanol and 20 % $H_2SO_4$ (1:1) ml of the filtrate was added to 5 ml of 60 % tetraoxosulphate (IV), and allowed to stand for 5 min. Then, 5 ml of 0.5 % formaldehyde was added and allowed to stand for 3 h. The reading was taken at absorbance of 565 nm [11].

#### Determination of saponins

Exactly 0.5 g of the sample was added to 20 ml of 1M NaCl and boiled for 4 hours. After cooling, it was filtered, and 50 ml of petroleum ether was added to the filtrate for ether layer formation and then evaporated to dryness. To the residue, 5 ml of acetone and ethanol was added. Then 6 ml of ferrous sulphate reagent was also added, followed by the addition of 2 ml of concentrated $H_2SO_4$. The solution was mixed for 10 min, and the absorbance was read at 490 nm [12].

#### Determination of tannins

Exactly 0.2 g of the extract was measured into a 50 ml beaker with 20 % methanol added and covered with para film then placed in a water bath at 77-80 $^{o}$C for one hour. Shake thoroughly to ensure a uniform mixing. The solution was filtered using a double layered whatman NO 41 filter paper into a 100 ml volumetric flask. Exactly 20 ml of water, 2.5 ml of folin-Ciocalteu reagent and10 ml of 17 % $Na_2CO_3$ were added and mixed properly. The mixture was made up to mark with water mixed and allowed to stand for 20 min. A bluish-green colour will develop at the end of range 0-10ppm. The absorbance of tannin acid standard solution and the sample were read after colour development on a spectrophotometer at wavelength of 760 nm [13].

### *In vitro* Antioxidant Assays

#### 1 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of the plant extracts was estimated using the DPPH radical scavenging assay as described by Zahra *et al*. [14]. Different concentrations of the samples (extracts, chitosan and the composite) and ascorbic acid (50, 100, 250, and 500 µg/ml) were prepared from stock solutions (1000 µg/ml) by weighing and dissolving 0.01 g of the samples and ascorbic acid (standard) in 10 ml of

methanol. Thereafter, 2 ml of 0.004 % DPPH in methanol was added to 1 ml of various concentrations of samples and ascorbic acid respectively. The reaction mixtures were incubated at 25 °C for 30 minutes. The absorbance of each test mixture was read against blank at 517 nm using double beam Shimadzu UV-1800 series spectrophotometer. The experiment was performed in triplicates. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ Inhibition} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} X100$$

### Ferric Reducing Antioxidant Power (FRAP) Assay

Estimation of antioxidant activity of the plant extracts via ferric reducing antioxidant power assay was conducted according to the method of Zahra *et al*. [14]. Stock solutions of samples (extracts, chitosan and their composite) and ascorbic acid (1000 µg/ml) were prepared from which different concentrations of 50,100, 250, and 500 µg/ml were prepared. In this assay, 1 ml of each plant extracts and ascorbic acid concentration was mixed with 1 ml of 0.2 M sodium phosphate buffer and 1 mL of 1 % potassium hexacyano ferrate (III). The reaction mixtures were incubated at 50 ºC for 20 minutes. Thereafter, 1 ml of 10 % TCA was added. The reaction mixtures were then centrifuged at for 10 minutes at room temperature. Then 1 ml of each supernatant obtained was mixed with 1 ml of distilled water and then 0.2 ml of 0.1% ferric chloride was added. The blank was prepared in the same extracts as samples except that the extracts were replaced by distilled water. The absorbance of the test mixtures was read at 700 nm. The percentage antioxidant activity was calculated using the formula below:

$$\% \text{ activity} = \frac{Asample - Ablank}{Asample} X100$$

## RESULTS

### Quantitative phytochemical constituents

The quantitative phytochemical constituents of ethanol extract of *T. procumbens* is as shown in Table 4.1. Phenols (439.96 $\pm$ 0.68 mg/100g) was significantly ($p<0.05$) higher in concentration when compared to the other phytoconstituents. However, this is followed by flavonoids (202.30$\pm$0.36 mg/100g), and then saponins (127.66 $\pm$ 0.60 mg/100g). Tannins content of *T. procumbens* extract was significantly ($p<0.05$) lower than the other phytoconstituents.

Table 1: Quantitative Phytochemical Composition of Ethanol Extract of *T. procumbens*

| Phytochemical constituents | Concentrations (mg/100g) |
|---|---|
| Phenols | $439.96 \pm 0.68^{e}$ |
| Flavonoids | $202.30 \pm 0.36^{d}$ |
| Tannins | $9.36 \pm 0.35^{a}$ |
| Saponins | $127.66 \pm 0.60^{c}$ |
| Alkaloids | $21.65 \pm 0.39^{b}$ |

Values are mean $\pm$ standard error of mean (SEM) of triplicate experiment. Values down the column with different letters as superscripts are considered significant at $p < 0.05$.

### cavenging activity of the plant extract on 2, 2-Diphenyl-2-Picryl Hydrazyl (DPPH) Radicals

The scavenging efficacy of ethanolic extract of *T. procumbens* on 2, 2-Diphenyl-2-Picryl Hydrazyl (DPPH) Radicals is presented in Table 2. The result revealed a concentration dependent DPPH scavenging activity of the samples and the standard. The highest percentage scavenging activity was observed at 500µg/ml while the

least was at 50 µg/ml. The scavenging activity of ascorbic acid (62.97±3.56 - 97.47±2.72 %) was significantly ($p<0.05$) higher than that of the ethanol extract of chitosan (29.87±0.32 - 57.05±0.55 %), *T. procumbens* extract (45.44±1.04 - 88.75±1.27 %) and their composite (57.21±1.19 - 91.65±0.58 %) at all the concentrations (50-500 µg/ml). Similarly, the scavenging activity of chitosan-*T. procumbens* extract composite was significantly ($p<0.05$) higher than that of the extract and chitosan alone

Table 2: DPPH Radical Scavenging Activity of Ethanol Extract *T. procumbens*

| Conc. (µg/ml.) | Ascorbic acid | T. p Extract | Chitosan | Cht-Tp |
|---|---|---|---|---|
| 50 | 62.97±3.56d | 45.44±1.04b | 29.87±0.32a | 57.21±1.19cd |
| 100 | 89.15±2.23d | 56.05±2.21b | 38.45±0.41a | 64.83±1.10c |
| 250 | 93.67±3.88d | 78.33±1.63b | 53.76±2.05a | 83.08±0.91c |
| 500 | 97.47±2.72d | 88.75±2.07b | 57.05±0.55a | 91.65±0.58bcd |

Values are mean ± standard error of mean (SEM) of triplicate experiment. Values across the rows with different letters are considered significant at $p < 0.05$. Key: T.p Extract = *T. procumbens* extract, Cht-Tp = Chitosan- *T. procumbens* extract composite

## Ferric Reducing Antioxidant Power (FRAP) of ethanolic extract of *T. procumbens*

The ethanol extract of *T. procumbens* demonstrated visible antioxidant effects in reducing $Fe^{3+}$ to $Fe^{2+}$ as shown in Table 3. The percentage of inhibition of ascorbic acid, ethanol extract of *T. procumbens,* chitosan and the composite of chitosan- *T. procumbens* extract increases with increase in concentration. Although, the ascorbic acid showed significantly ($p<0.05$) higher FRAP scavenging activity at all the concentrations than the other samples. However, the chitosan-*T. procumbens* extract composite had % inhibition (64.81±0.87 and 90.65±0.11 %) comparable ($p>0.05$) to ascorbic acid standard (71.09±0.88 and 96.69±3.67 %) at concentrations of 50 and 500 µg/ml respectively. Chitosan had significantly lower ($p>0.05$) scavenging capacity when compared to the other and standard at all the concentrations

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Table 3: Ferric Reducing Antioxidant Power of Chitosan and ethanol extract of *T. procumbent* Composite

| Conc. (µg/ml.) | Ascorbic acid | T. p Extract | Chitosan | Cht-Tp |
|---|---|---|---|---|
| 50 | 71.09±0.88d | 33.82±0.75a | 38.46±1.08b | 64.81±0.87cd |
| 100 | 82.29±1.02d | 52.65±2.16b | 49.71±1.44a | 76.18±1.49c |
| 250 | 90.28±1.35d | 77.12±1.51b | 68.25±1.39a | 83.10±2.03c |
| 500 | 96.69±3.67c | 82.72±1.88a | 79.87±0.76a | 90.65±0.11c |

Values are mean ± standard error of mean (SEM) of triplicate experiment. Values across the rows with different letters as superscripts are considered significant at $p < 0.05$. Key: T.p Extract = *T. procumbens* extract, Cht-Tp = Chitosan- *T. procumbens* extract composite

## DISCUSSIONS

The presence of phenols, flavanoids, saponins, alkaloids, and tannins found in the ethanol extract of *T. procumbens* agrees with the findings of Nakum *et al.* [15] who evaluated the *in-vitro* antioxidant activities and phytochemical analysis in different solvents extracted from *T. procumbens*. The significantly high phenols concentration in *T. procumbens* is agreement with study of Akinola and Adelowo [16] who evaluated some phenolic compounds in *T. procumbens* by chromatographic and spectrophotometric methods. However, this is in disagreement to the findings of Nakum *et al.* [15] which states that saponins was the major

phytoconstituents in *Tridax procumbens.* This variation in phytochemical concentration of *T. procumbens*, maybe as a result of difference in species, part of the plant being used for the analysis and method of extraction.

The observed scavenging activity of ethanol extract of *Tridax procumbens* could be explained by the presence of different phyto compounds found in the plant and this agrees with the findings of Singh *et al*. [17]; Nakum *et al*. [15] and Andriana *et al*. [18]. Earlier researchers reported that phenols and flavanoids contents of various plants extract are directly responsible for their antioxidant activities. So, the higher concentration of phenols and flavanoids in ethanol extract of *Tridax procumbens* maybe responsible for the elicited antioxidant effect. This may be due to the rich hydroxyl (-OH) groups in polyphenols that can donate hydrogen atom(s) to free radicals (e.g., reactive oxygen species like superoxide anion, hydroxyl radical) to produce a more stable molecule and a resonance-stabilized radical, which is much less reactive. Flavanoids usually chelate trace elements involve in free-radicals production and suppress reactive oxygen formation, scavenge reactive species. Similarly, phenolic compounds confer oxidative stress tolerance on plants [19].

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay is one of the most employed methods to evaluate the free radical scavenging efficiency of antioxidant compounds. This assay is based on the reduction of the DPPH radical (deep purple) to a non-radical form (yellow) in the presence of hydrogen-donating antioxidants. The degree of discoloration reflects the scavenging ability of the antioxidant.

In the current study, the ethanol extract of *Tridax procumbens* exhibited strong DPPH radical scavenging activity, aligning with earlier reports highlighting its rich phytochemical content. Studies by Bhaskar et al. [20] and Nariya *et al.* [21] have shown that *T. procumbens* is a potent source of flavonoids, tannins, and phenolic acids, which are effective hydrogen donors capable of neutralizing free radicals [20, 21]. The presence of quercetin, catechins, and other polyphenols likely contributes to this robust antioxidant capacity.

Chitosan, by contrast, demonstrated comparatively lower DPPH scavenging activity. Although chitosan has some intrinsic antioxidant properties due to its free amino groups, which can interact with and stabilize free radicals, its performance in DPPH assays is generally moderate and varies with molecular characteristics. Wang *et al.* [23] and Yen *et al.* [24] found that low molecular weight and high degree of deacetylation in chitosan improve its radical scavenging properties, but overall, it is not as effective as polyphenol-rich plant extracts in this context [23, 24].

The composite of chitosan with *T. procumbens* extract showed a significantly enhanced DPPH radical scavenging activity compared to both the extract or chitosan alone and was comparable to the standard ascorbic acid. This enhancement could be attributed to the stabilization of phenolic compounds by the chitosan matrix, which may protect these bioactives from degradation and enhance their sustained release. Additionally, the electrostatic interactions between chitosan's amine groups and the hydroxyl groups of polyphenols may create a microenvironment favorable for antioxidant activity [25].

Moreover, the composite formulation could mitigate the volatility (especially for low molecular weight phenols), degradation and oxidative sensitivity of certain phytochemicals, thereby improving their radical scavenging potential over time. Similar findings have been observed in other biopolymer–phytochemical systems, such as chitosan-green tea or chitosan-turmeric composites, which show increased antioxidant performance due to synergistic interactions between polymer and plant bioactives [26].

The Ferric Reducing Antioxidant Power (FRAP) assay is a widely accepted method for evaluating the total antioxidant potential of plant extracts and biomaterials. It quantifies the ability of antioxidants to reduce $Fe^{3+}$ (ferric) to $Fe^{2+}$ (ferrous) ions, which reflects the electron-donating capacity of bioactive compounds. In the present study, we assessed the FRAP activity of ethanol extract of *Tridax procumbens*, pure chitosan, and their formulated composite.

The ethanol extract of *T. procumbens* demonstrated significant ferric reducing capacity, consistent with previous studies reporting its rich content of polyphenols, flavonoids, and other redox-active constituents. For instance, Nariya *et al.* [22] documented high total phenolic content and substantial antioxidant activity in ethanolic extracts of *T. procumbens*, attributing this to the presence of compounds such as quercetin, luteolin, and tannins, which are known for their efficient electron transfer mechanisms [22]. These phytochemicals act as primary antioxidants by donating electrons to neutralize free radicals or reduce oxidized intermediates.

Chitosan, on the other hand, exhibited moderate FRAP activity. While chitosan is not a strong antioxidant on its own, it possesses inherent reducing capacity due to the presence of amino groups, which can scavenge free radicals and reduce metal ions to some extent. Yen *et al.* [21] reported that the antioxidant activity of chitosan is influenced by factors such as its molecular weight and degree of deacetylation, with low molecular weight chitosan showing enhanced ferric reducing power.

Interestingly, the chitosan-*T. procumbens* composite exhibited a synergistically enhanced FRAP value compared to the individual components. This increase suggests a potential interaction between the polyphenolic compounds of *T. procumbens* and the chitosan matrix that may stabilize active antioxidant compounds and enhance their availability. Similar findings have been reported in composites of chitosan with other plant extracts, where encapsulation or entrapment in the chitosan matrix preserved or even enhanced the antioxidant activity of the incorporated bioactives [25].

The observed enhancement in antioxidant capacity in the composite may be attributed to the sustained release of phytochemicals from the chitosan matrix, the protective effect of chitosan against degradation of sensitive antioxidant compounds, and the potential hydrogen bonding or electrostatic interactions that stabilize active species.

## CONCLUSION

Summary, *T. procumbens* have a good antioxidant activity but the combination of both *T. procumbens* and chitosan had a better antioxidant property, making it a promising candidate for biomedical or food packaging applications where oxidative stability is crucial.

### Authors Contribution

OIF conceptualized the study. OIF, MHK and SBS designed the study. DEA, UIO, SOD and OMO participated in laboratory work. UAC performed the data analysis; OIF, UAC and OOA interpreted the data. DEA, UIO, SOD and OMO prepared the first draft of the manuscript, reviewed by OIF, UAC and OOA. All authors contributed to the development of the final manuscript and approved its submission.

## Conflict of Interest

There is no conflict of interest among the authors

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