Original Article

Exploring the Antioxidant Potential of a Polyherbal Combination: A Comprehensive *in vitro* Study

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ABSTRACT

Background: The objective of this research was to explore the antioxidant potential of the three different medicinal plant extracts namely *Curcuma longa, Acacia catechu* and *Allium cepa* along with their combination (polyherbal) as well as to evaluate their phytochemicals, physical parameters, quantitative analysis of flavonoids, phenolic contents. **Materials and Methods:** The three plants' ethanolic extracts were collected using the soxhlet apparatus. The phytochemicals of the plant extracts were analyzed using different analytical reagents. Physical parameters such as extractive value, Loss on Drying (LOD), and ash values have been studied. The Folin-Ciocalteu phenol reagent was used to assess the phenolic content, and the aluminium chloride colorimetric technique was used to calculate the flavonoid level. The antioxidant activity of the individual plant extract and the polyherbal extracts (10 µL each extract) was determined using the DPPH, ABTS, hydroxy free radical scavenging assay, CUPRAC, FRAP, and superoxide anion radical scavenging assay. **Results:** The phytochemical tests revealed the common presence of flavonoids, tannins, and glycosides in *C. longa, A. catechu* and *A. cepa*. The extractive value was calculated as 24.90 g, 6.93 g, and 30.71 g, for *C. longa, A. catechu*, and *A. cepa* respectively. The total ash (%w/w), acid-insoluble ash (%w/w), and water-soluble ash (%w/w) values for *C. longa* were 13.21, 1, and 4.13, respectively, for *A. cepa* was 2.59, 0.28, and 1.09, respectively, and for *A. catechu* was 12.6, 1.63, and 6.25, respectively. LOD (%w/w) for *C. longa, A. cepa*, and *A. catechu* was found to be 4.09, 10.59, and 7.23, respectively. The total phenolics content was found to be 56.08±0.23 mg GAE/g, 39.7±0.20 mg GAE/g, and 16.0±0.2 mg GAE/g in *C. longa, A. catechu*, and *A. cepa*, respectively. Total flavonoid content was calculated as 19.1±0.3 mg QE/g, 9.12±0.3 mg QE/g, and 53.89±0.30 mg QE/g in *C. longa, A. catechu*, and *A. cepa*, respectively. All the extracts including the polyherbal combination showed significant antioxidant activity by assessing their IC_{50} value. **Conclusion:** The superoxide anion radical scavenging assay verifies the robust efficacy of the polyherbal extract and proposes it as a specific antioxidant drug against disorders associated with excessive superoxide radical production like wound, atherosclerosis, amyotrophic lateral sclerosis, cancer progression, rheumatoid arthritis, accelerated aging, etc.

Keywords: Polyherbal combination, Phytochemical analysis, Antioxidant activity, DPPH, ABTS, Hydroxy free radical scavenging assay, CUPRAC, FRAP, Superoxide anion radical scavenging assay.

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INTRODUCTION

The idea of polyherbalism in Ayurveda is that Phyto compounds from various plants are more medicinal than extracts from a single plant. The efficacy in different medical and therapeutic contexts such as extracellular matrix remodelling, angiogenesis, antioxidant activity, and wound healing, has gained international recognition for polyherbal formulations (V Rastogi, M Porwal, n.d.). In polyherbal medications, several different herbs are mixed in some specified ratio to increase therapeutic benefits and reduce toxicity (Azhar, 2007).

The active compound in the rhizome of the plant *Curcuma longa* of the family Zingiberaceae is curcumin which is used widely. The polyphenolic compound curcumin has potent antioxidant, antimicrobial, and antibacterial properties and can therefore contribute to antioxidant activity (Salehi *et al*., 2021). Catechin is found in *Acacia catechu*, a species of the Fabaceae family derived from the bark of the acacia tree. Catechin is essential for enhancing antioxidant activity (Stohs & Bagchi, 2015). *Allium cepa*, a species of the Liliaceae family, is a bulbous onion characterized by its antioxidant characteristics, which include the natural flavonoid quercetin (Kant *et al*., 2021). The evidence suggests that the use of

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polyherbal combinations, incorporating several compounds, such as quercetin, curcumin, and catechin, might achieve a synergistic antioxidant activity greater than could be achieved by any given combination agent singly. This synergistic strategy couples the unique, and complementary, processes of each component to achieve more robust therapeutic effects.

An antioxidant is a substance that prevents oxidation-a chemical process that generates free radicals which are very reactive molecules that can damage DNA, proteins, and cells (Rastogi *et al*., 2023). Free radicals are a normal result of metabolism but can be produced by exposure to UV light, pollution, or injury. The antioxidants lower oxidative stress in the body by scavenging these free radicals (Pham-Huy *et al*., 2008).

The initial inflammatory phase produces free radicals as part of the body's immunological response and free radicals that are part of the body's response to remove damaged tissue. Too many free radicals can also harm healthy cells and slow the healing process. Antioxidants are those that help to balance this reaction because they decrease oxidative stress and enhance healthy immunological function (Tumilaar *et al*., 2023).

Other well-known antioxidants, including flavonoids, coenzyme Q10, and vitamins C and E, also improve the suppleness of the skin and help improve the overall healing results (Raina *et al*., 2021).

MATERIALS AND METHODS

Collection and Authentication of Plants Materials

In July 2023, *Acacia catechu*'s bark, *Curcuma longa*'s rhizomes, and *Allium cepa*'s bulb were gathered from the local market in Amroha, India. Dr. S. Garg at CSIR-NISCAIR, Delhi verified the authenticity of the materials using the following authentication numbers: NIScPR/RHMD/Consult/2023/4527-28-1, NIScPR/RHMD/Consult/2023/4527-28-2, and NIScPR/RHMD/Consult/2023/4527-28-3. After being bought from the same location in Amroha, the necessary quantity of authenticated plant onion bulbs, rhizomes, and bark were cleaned, cut, and dried for seven days under a sunshade. Following drying, the onion bulb, rhizomes, and bark were crushed coarsely and sieved through sieve number 60 and kept out of direct sunlight in an airtight container (*The Ayurvedic Pharmacopoeia of India, Development and Perspectives*, 2017).

Preparation of individual Plant Extracts

The soxhlet extraction method was used to extract various plant extracts from different plants. The first step involved defatting of *Curcuma longa* rhizomes (300 g), *Acacia catechu* bark (200 g), and *Allium cepa* bulb (100 g) with petroleum ether at 60-80ºC, followed by extraction with 600 mL of ethanol for 6 hr. Excess solvents were collected using reflux condensation, and the resulting extracts were filtered and concentrated at 40ºC using a rotary evaporator (Grover; Madhuri *et al*., 2021; Nakhate *et al*., 2023; Saptarini & Wardati, 2020).

The following formula determined the percentage yield of each of the three plant extracts:

$$\% \text{ Yield of extract} = \frac{\text{Weight of crude}}{\text{Weight of the sample}} \times 100$$

Screening Test for Phytochemicals

The study assessed various phytoconstituents in three crude extracts: proteins, alkaloids, glycosides, terpenes, carbohydrates, flavonoids, phenol, tannins, saponins, and steroids. These substances have various biological functions, including anxiolytic, antioxidant, anti-inflammatory, pain-relieving, wound-healing, anticancer, and antihypertensive effects. The phytochemical testing involved combining extracts with diluted HCl, detecting orange-red precipitates, carbohydrates, glycosides, saponins, steroids, and biuret reagent (Mankad *et al*., 2018). Protein screening tests revealed proteins as a bluish-violet tint, terpenoids as reddish-brown, flavonoids as yellow, anthraquinones as pink to red, phlobatannins as red precipitates, tannins as dark green, amino acids as purple, steroids as blue, and phenols as reddish-brown precipitates (Pawar, 2014).

The presence of these substances was confirmed through various tests, including the presence of anthraquinones, phlobatannins, tannins, amino acids, steroids, and phenols. The presence of a violet ring, a pink colour development, a foamy layering, and a reddish-brown precipitate confirmed the presence of these substances. The results of these tests provide valuable insights into the biological properties of the extracts (Kumar Gupta *et al*., 2019).

Physicochemical analysis of extracts

Determination of Loss on Drying

After weighing approximately 1.5 g of each of the three extracted drugs powdered onto a tarred porcelain dish, heat it at 100ºC or 105ºC to dry it. It was then allowed to cool in desiccators, and the weight loss was detected as moisture (Lakshmi *et al*., 2017). The loss on drying for all the three plant extracts was calculated using the formula below:

$$\%\text{Loss on drying (LOD)}=\frac{\textit{Initial weight of the sample} - \textit{Weight of sample after drying}}{\textit{Initial weight of the sample}} \times 100$$

Determination of Total ash value

A tarred silica crucible was precisely filled with 2 g of each of the three extracted powdered drugs. At 450ºC, it was burnt in a muffle furnace until all of the carbon-free. Weighing was done once the crucible had cooled. The air-dried material was taken into account when calculating the percentage of total ash (Lakshmi *et al*., 2017). The following formula was applied to determine the total ash value:

$$\text{Total ash value of the sample}=\frac{\textit{Ash weight}}{\textit{Weight of sample}} \times 100$$

Determination of Acid Insoluble ash

After boiling the ash from all three extracted powdered drugs for a few minutes with 25 mL of 2N HCl, the ash was filtered through ash-less filter paper. The filter paper was supported by a tarred silica crucible and burnt in a muffle furnace at 450ºC until it was carbon-free. When the crucible had cooled, it was weighed. The proportion of acid-insoluble ash for each of the three extracted powdered drugs was calculated for each air-dried material (Lakshmi *et al.*, 2017). The value of acid-insoluble ash was determined using the formula below:

$$\text{Acid Insoluble ash}=\frac{Acid\ insoluble\ Ash\ weight}{Weight\ of\ sample}\times 100$$

Determination of Water-soluble Ash

All three extracted powdered drugs (also powdered ash-free filter paper) were boiled briefly in 25 mL distilled water and then filtered through ashless filter paper. A carbon-free filter paper was produced by burning the tarred silica crucible containing the filter paper at 450ºC in a muffle furnace. After it had cooled, the crucible was weighed. Proportions of water-soluble ash for all three extracted powdered drugs were determined to be air-dried (Lakshmi *et al.*, 2017). The determination formula for water-soluble ash value was:

$$\text{Water soluble ash}=\frac{Total\ Ash\ weight-water\ insoluble\ residue\ in\ total\ ash}{Weight\ of\ sample}\times 100$$

Estimation of Total Phenolic Content (TPC)

The phenolic acid content of ethanolic extract of *Curcuma longa, Acacia catechu*, and *Allium cepa* was estimated using the Folin Ciocalteu technique. The measured concentration was 1 mg/mL. After weighing 10 mg of the test extracts, they were dissolved in 10 mL of ethanol, the solvent. 1 mL of plant extracts (1 mg/mL) and 5 mL of distilled water were mixed with 1 mL of Folin Ciocalteu's phenol, then 1 mL of 10% (w/v) Na_2CO_3 solution was added, and left for an hour. A UV-visible spectrophotometer was used in a later step to detect the absorbance at 750 nm. The gallic acid standard calibration curve made up the basis for the calculation, thus TPC was expressed as mg GAE/g of the extract. Every experiment was run in triplicate (*n*=3) (Pandey & Sharma, 2022).

Estimation of Total Flavonoid Content (TFC)

The flavonoid content of the *Curcuma longa, Acacia catechu*, and *Allium cepa* ethanol extract has been assessed using the Aluminium chloride colorimetric technique. This method involved mixing 4 mL of distilled water with 1 mL of extract (1 mg/mL) and then adding 0.3 mL of a 5% Sodium nitrite solution. 0.3 mL of 20% Aluminium chloride is added, and it is then let to settle for 6 min. 2 mL of Sodium hydroxide (1 M) was further added. A UV-visible spectrophotometer was used to determine the resulting mixed solution's absorbance at 420 nm. Using a quercetin calibration curve, the TFC-which is expressed as mg QE/g of the extract-was determined (Pandey & Sharma, 2022). The run was made in triplicate (*n*=3) for every experiment.

In vitro antioxidant activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) Scavenging Assay

5 μL of various test chemical stocks. O1 extract from *Curcuma longa*, O2 extract from *Acacia catechu*, O3 extract from *Allium cepa*, and O4 extract-a combination of all three extracts (10 μL each extract)-were combined in a 96-well plate with 0.1 mL of a 0.1 mM DPPH solution. The reaction was set up in triplicate, and duplicate blanks were made with 5 microliters of a chemical at varying concentrations and 0.2 μL of DMSO/methanol. Wells without reagent (DPPH) were referred to as blanks, while wells without treatment were referred to as controls. The plate was kept in the dark for half an hour. At 517 nm, the decolorization was detected using a microplate reader. As a control, 20 μL of deionized water was added to the reaction mixture. The scavenging activity was controlled and displayed as "% inhibition." The sample concentration (X-axis) and the % inhibition relative to control (Y-axis) were shown on a graph (Imam *et al.*, 2011). Equation 1 was used to determine the concentrations of 10 μL of various stocks of the standard (Ascorbic acid) and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts.

Calculations

$$\%RSA=\frac{((Abs\ control-Abs\ sample)}{Abs\ control}\times 100\ [1]$$

% RSA=((Abs Control-Abs Sample)/Abs Control) ×100

RSA=Radical Scavenging Activity,

$Abs_{Control}$=Absorbance of control,

Abs_{Sample}=Absorbance of sample.

ABTS (2,2′-azino-bis-(3-ethylbenzothiazoli ne-6-sulfonic acid) Radical Scavenging Ability

ABTS (SRL-Chem-Cat no.-28042) radicles were created by combining ABTS (7 mM) and APS (2.45 mM) solution, which had been diluted 100X to make ABTS free radical reagent. In 96-well plates, 200 μL of ABTS free radical reagent was mixed with 10 μL of various stock of standard (ascorbic acid) and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts. The untreated wells served as the control group. Post incubation, measure the decolorization's absorbance at 750 nm using a microplate reader. The results were shown to the negative control group. Software Graph Pad Prism 9.5.1 was used to determine the IC_{50}. The X-axis (sample concentration) and the Y-axis (% inhibition relative to control) were used to create the graph (Kambayashi *et al.*, 2009).

Equation 1 was used to determine the concentrations of 10 μL of various stocks of the standard and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts.

Hydroxy Free Radical Scavenging Assay

In the wells of a 96-well plate, 66 μL of the Reagent Mixture (0.5 M), 24.14 mg Deoxyribose, 88 μL $FeCl_3$ (10 mg/mL), 28 μL H_2O_2 (6%), water up to 33 mL, 10 μL of plant extracts O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts, 10 μL of ascorbic acid and 24 μL of phosphate buffer (50 mM, pH 7.4). After that, the mixture was incubated at 37ºC for one hour. The untreated wells served as the control group. Gallic acid was the standard utilized. Following incubation, each well received 50 μL of 10% TCA and 50 μL of 1% TBA. The chromogen became pink. The absorbance at a wavelength of 540 nm was measured using a microplate reader. The IC_{50} was determined using GraphPad Prism 6. The X-axis (sample concentration) and the Y-axis (% inhibition relative to control) were used to create the graph (Rahman *et al*., 2015). Equation 1 was used to determine the concentrations of 10 μL of various stocks of the standard and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts.

Total Antioxidant Assay-CUPRAC (Cupric reducing antioxidant capacity) Assay

10μL of the test samples at various concentrations in designated wells of a 96-well plate, O1 extract from *Curcuma longa*, O2 extract from *Acacia catechu*, O3 extract from *Allium cepa*, and O4 combination of all three extracts and standard were added. 200 μL of the reagent combination was then added to a 96-well plate. 200 μL of methanol and 10 μL of a component with varying quantities for plant extracts and their combination and standard (Trolox-Ottokemi-Cat no.-T7723) were made for the reaction mixture in triplicate form and the blank in duplicate form. The untreated wells served as the control group. After incubation, the absorbance of the decolorization at 490 nm was measured using a microplate reader. The control was a reaction mixture in which the sample or standard was substituted with 20 μL of deionized water. To control, scavenging activity was displayed as "% inhibition." The IC_{50} was determined using GraphPad Prism 6. The X-axis (sample concentration) and the Y-axis (% inhibition relative to control) were used to create the graph (Rubio *et al*., 2016). Equation 1 was used to determine the concentrations of 10 μL of various stocks of the standard (Trolox-Ottokemi-Cat no.-T7723) and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts.

FRAP (Ferric reducing antioxidant power)

0.04 mL of a pH 6.6 buffer containing 0.2 M sodium phosphate and 0.05 mL of 1% potassium ferricyanide $[K_3Fe (CN)_6]$ solution was mixed with 10 μL of various stocks of the test compounds O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts and standard (ascorbic acid). After thoroughly vortexing the reaction mixture, it was incubated for 20 min at 50ºC. The untreated wells served as the control group. The mixture was mixed with 0.5 mL of 10% trichloroacetic acid following the incubation time. 50 μL of 0.1% ferric chloride and 50 μL of deionized water were then added. A microplate reader was used to read the colored solution at 700 nm in comparison to the blank. Calculating the IC_{50} was done using Graph Pad Prism 6 (Rao *et al*., 2013). Equation 1 was used to determine the concentrations of 10 μL of various stocks of the standard and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts.

Super Oxide Anion Radical Scavenging Assay

The riboflavin solution was supplemented with varying quantities of the test substances O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts, as well as the standard (gallic acid). This combination was incubated in a 96-well plate for 30 min at room temperature with light. Post incubation, the reaction mixture was added to the mixture that had already been incubated and properly mixed. The untreated wells were regarded as managed. An Elisa plate reader was then used to measure the absorbance at 560 nm. The IC_{50} was determined using GraphPad Prism 6. The X-axis (sample concentration) and the Y-axis (% inhibition relative to control) were used to create the graph (Noda *et al*., 1997). Equation 1 was used to determine the concentrations of 10 μL of various stocks of the standard and test samples O1 *Curcuma longa* extract, O2 *Acacia catechu* extract, O3 *Allium cepa* extract, and O4 combination of all three extracts.

RESULTS

Percentage yield and extractive value

The % yield of crude drug extract for *Curcuma longa, Allium cepa*, and *Acacia catechu* was found to be 8.30%, 30.71%, and 3.46%, respectively. At the same time, the extractive value was calculated as 24.90 g, 30.71 g, and 6.93 g, respectively.

Phytochemical Screening of extracts

The phytochemical tests were performed for all three extracts and results were presented in Table 1.

Physicochemical analysis of extracts

The total ash (%w/w), acid-insoluble ash (%w/w), and water-soluble ash (%w/w) values for *Curcuma longa* were

13.21%w/w, 1%w/w, and 4.13%w/w, respectively for *Allium cepa* it was 2.59%w/w, 0.28%w/w, and 1.09%w/w, respectively, and for *Acacia catechu* it was 12.6%w/w 1.63%w/w, and 6.25%w/w, respectively. The Loss on drying (%w/w) for *Curcuma longa, Allium cepa*, and *Acacia catechu* was found to be 4.09%w/w, 10.59%w/w, and 7.23%w/w, respectively.

Estimation of Total Phenolic Content

The standard calibration for standard gallic acid is represented in Figure 1, the relationship (y=0.184x + 0.079, R^2=0.998) was utilized to determine the (TPC) of the plant extracts. The TPC of plant extracts from *Curcuma longa, Acacia catechu*, and *Allium cepa* was 56.08±0.23 mg GAE/g, 39.7±0.20 mg GAE/g, and 16.0±0.2 mg GAE/g dry weight.

Estimation of Total Flavonoid Content

Figure 2 represents the standard calibration curve for quercetin. The relationship (y=0.154x + 0.168, R^2=0.999) was used for calculating the TFC present in extracts. It was found that the TFC of the *Curcuma longa, Acacia catechu*, and *Allium cepa* plant extract was 19.1±0.3 mg QE/g, 9.12±0.3 mg QE/g, and 53.89±0.30 mg QE/g dry weight.

In vitro Antioxidant activity assay

2,2-diphenyl-1-picrylhydrazyl (DPPH) Scavenging Assay

Based on the results obtained from the experimental work, antioxidant activity (DPPH Assay) was estimated in samples, and 50% Inhibitory Concentration (IC_{50}) was mentioned in Figure 3. Sample O2 (*Acacia catechu* extract) was found to be the most active among all the samples. The IC_{50} values for samples

Table 1: Phytochemical Screening of extracts.

Test	*Curcuma longa* Ethanolic extract	*Allium cepa* Ethanolic extract	*Acacia catechu* Ethanolic extract
Saponins	+	-	+
Tannins	+	+	+
Phenol	+	-	+
Carbohydrates	+	-	+
Proteins	+	-	-
Amino Acids	+	-	+
Flavonoids	+	+	+
Phlobotannins	+	-	-
Alkaloids	+	-	+
Glycosides	+	+	+
Steroids	+	-	-
Anthraquinones	+	-	-
Terpenoids	+	-	-

+ indicates=Presence;-indicates=Absence.

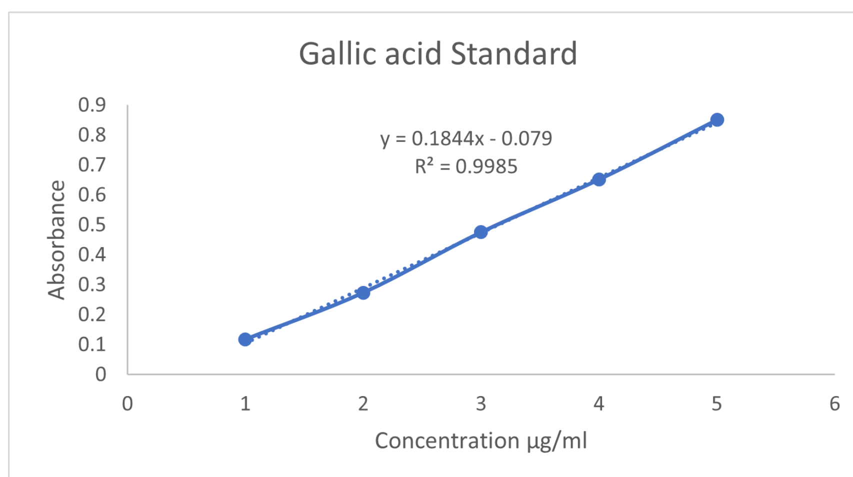

Figure 1: Calibration curve of standard gallic acid.

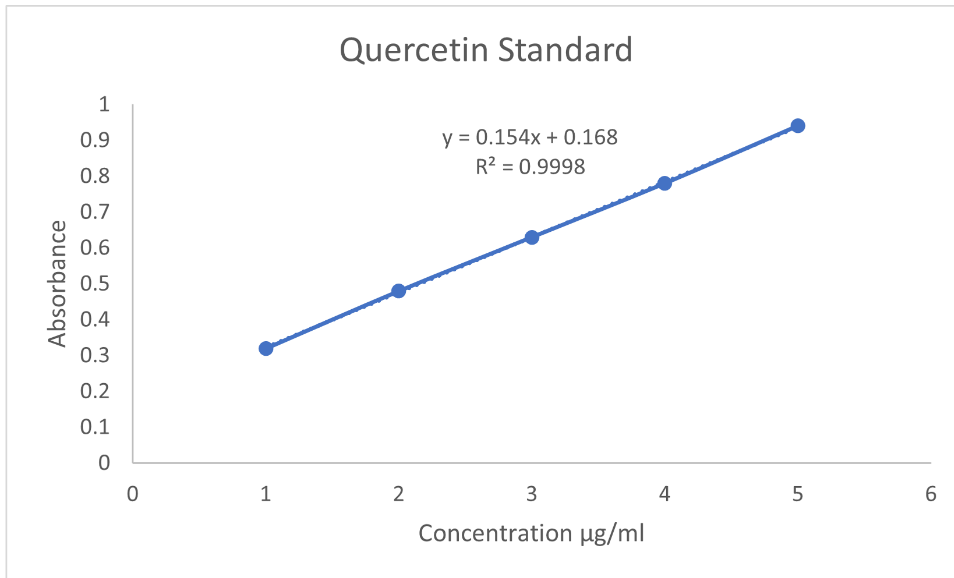

Figure 2: Calibration curve of standard quercetin.

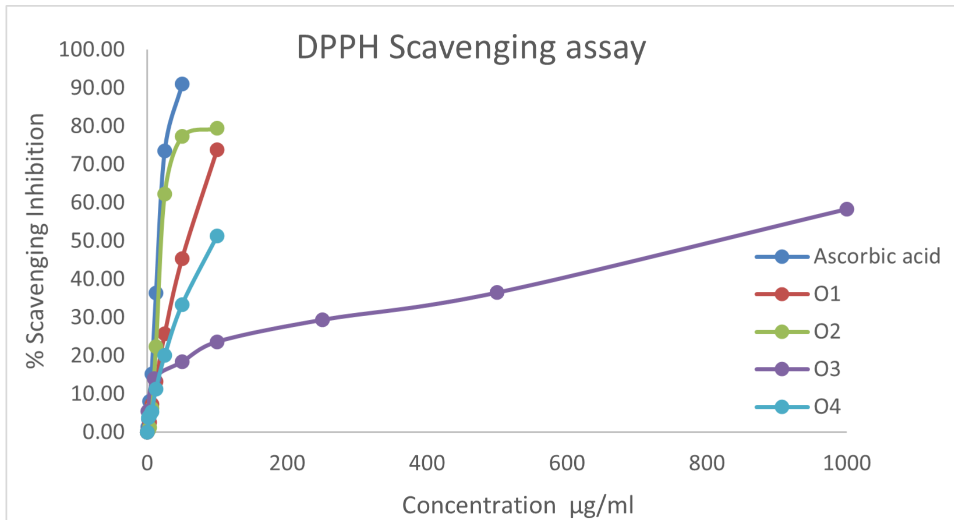

Figure 3: DPPH scavenging assay of Ascorbic acid, O1, O2, O3, and O4.

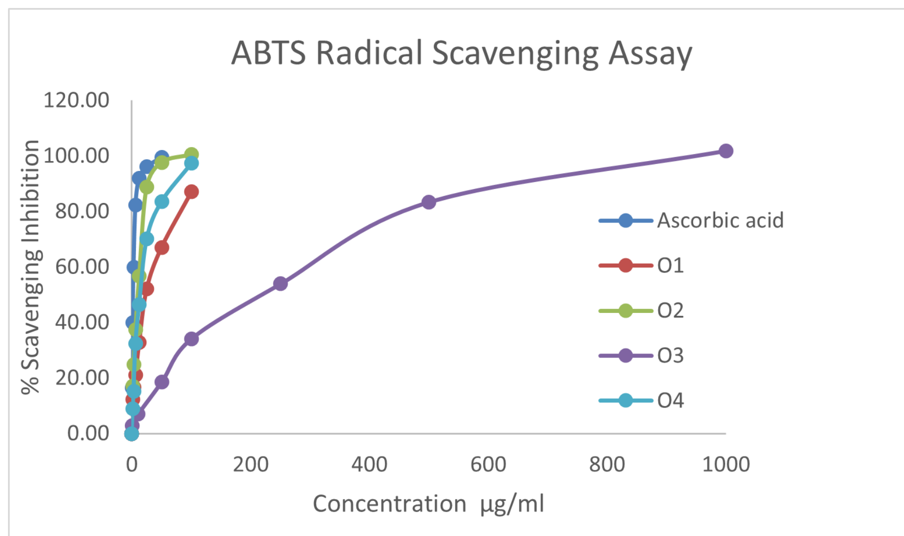

Figure 4: ABTS Radical Scavenging Ability of Ascorbic acid, O1, O2, O3, and O4.

O1 (*Curcuma longa* extract), O2 (*Acacia catechu* extract), O3 (*Allium cepa* extract), and O4 (Combination of all three extracts) were 52.47, 23.31, 943.3, and 97.32 µg/mL, respectively, which is equivalent to 15.59 µg/mL of standard ascorbic acid.

ABTS Radical Scavenging Ability

Based on the results obtained from the experimental work, antioxidant activity (ABTS Radical Scavenging Assay) was estimated in samples, and a 50% inhibitory concentration was mentioned in Figure 4. Sample O2 (*Acacia catechu* extract) was found to be the most active among all the samples. 22.24 µg, 8.102 µg, 171.9 µg, and 12.54 µg of samples O1 (*Curcuma longa* extract), O2 (*Acacia catechu* extract), O3 (*Allium cepa* extract), and O4 (Combination of all three extracts), respectively, were found equivalent to 2.233 µg of standard Ascorbic acid.

Hydroxy Free Radical Scavenging Assay

Based on the results obtained from the experimental work, antioxidant activity (Hydroxy Free Radical Scavenging Assay) was estimated in samples, and 50% inhibitory concentration was mentioned in Figure 5. Sample O1 (*Curcuma longa* extract) was found to be the most active among all the samples. 2.147 µg, 45.36 µg, 9.039 µg, and 3.839 µg of samples O1 (*Curcuma longa* extract), O2 (*Acacia catechu* extract), O3 (*Allium cepa* extract), and O4 (Combination of all three extracts) were found to be equivalent to 5.462 µg of standard gallic acid.

Total Antioxidant Assay-CUPRAC Assay

Based on the results obtained from the experimental work, antioxidant activity (CUPRAC Assay) was estimated in samples, and 50% inhibitory concentration was mentioned in Figure 6. Sample O1 (*Curcuma longa* extract) was the most active among all the samples for the CUPRAC assay. 2.629 µg, 7.743 µg, 6.331 µg, and 4.693 µg of samples O1 (*Curcuma longa* extract), O2 (*Acacia catechu* extract), O3 (*Allium cepa* extract), and O4 (Combination of all three extracts), respectively, were found equivalent to 5.696 µg of standard Trolox.

Total Antioxidant Assay-FRAP

Based on the study's results, antioxidant activity (FRAP) was estimated in samples, and 50% inhibitory concentrations were mentioned in Figure 7. Sample O2 (*Acacia catechu* extract) was found to be the most active among all the samples. 8.999 µg, 6.898 µg, 51.91 µg, and 8.485 µg of samples O1 (*Curcuma longa* extract), O2 (*Acacia catechu* extract), O3 (*Allium cepa* extract), and O4 (Combination of all three extracts), respectively, were found equivalent to 4.312 µg of standard Ascorbic acid.

Super Oxide Anion Radical Scavenging Assay

As per results obtained from the experimental work, antioxidant (Super Oxide Anion Radical Scavenging) was estimated in samples and 50% Inhibitory Concentration (IC_{50}) was mentioned in Figure 8. Sample O4 (combination of all three extracts) was found to be the most active among all the samples. 124.7 µg, 27.41 µg, 56.55 µg and 16.3 µg of samples O1 (*Curcuma longa* extract), O2 (*Acacia catechu* extract), O3 (*Allium cepa* extract), and O4 (Combination of all three extracts), respectively were found equivalent to 38.17 µg of standard Gallic acid.

DISCUSSION

This study demonstrates the efficacy of polyherbal combinations comprising extracts of *Curcuma longa*, *Acacia catechu*, and *Allium cepa* in promoting antioxidant qualities. The polar bioactive chemicals were extracted utilizing the Soxhlet technique with ethanol as the solvent. Ethanol was selected because of its polarity, as it closely resembles the polarity of essential phytochemicals including curcumin, catechin, and quercetin, enhancing their extraction. The favorable yield and elevated extractive values

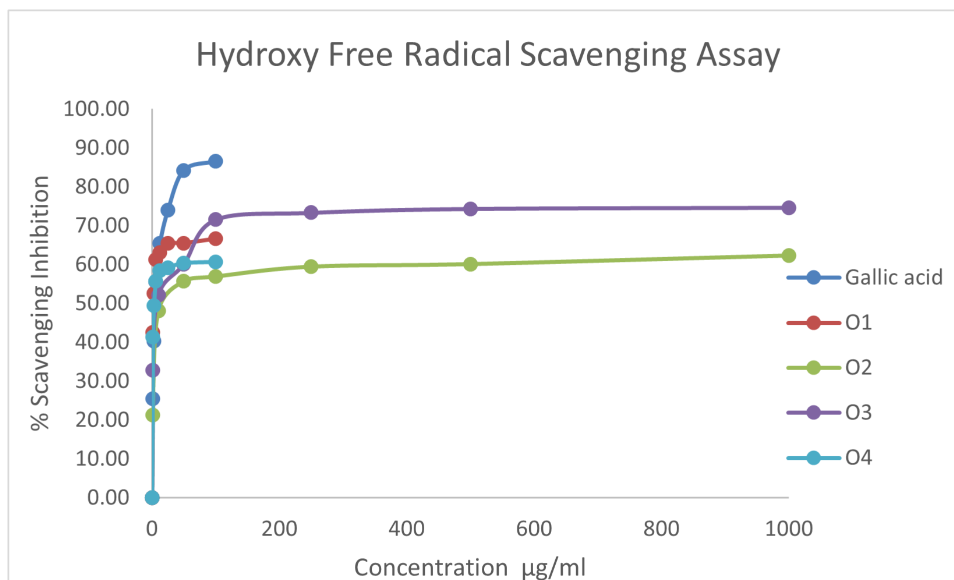

Figure 5: Hydroxy free radical scavenging assay of gallic acid, O1, O2, O3, and O4.

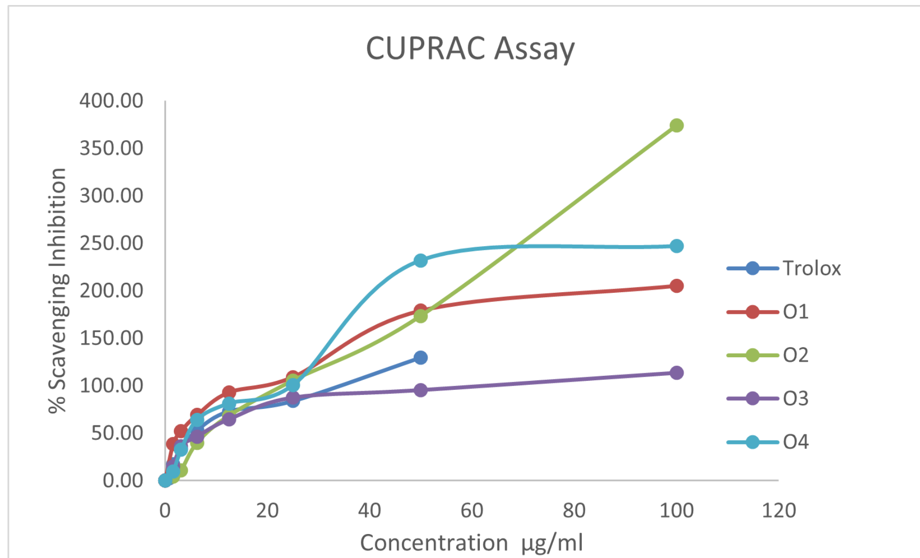

Figure 6: Total Antioxidant Assay-CUPRAC Assay of Trolox, O1, O2, O3, and O4.

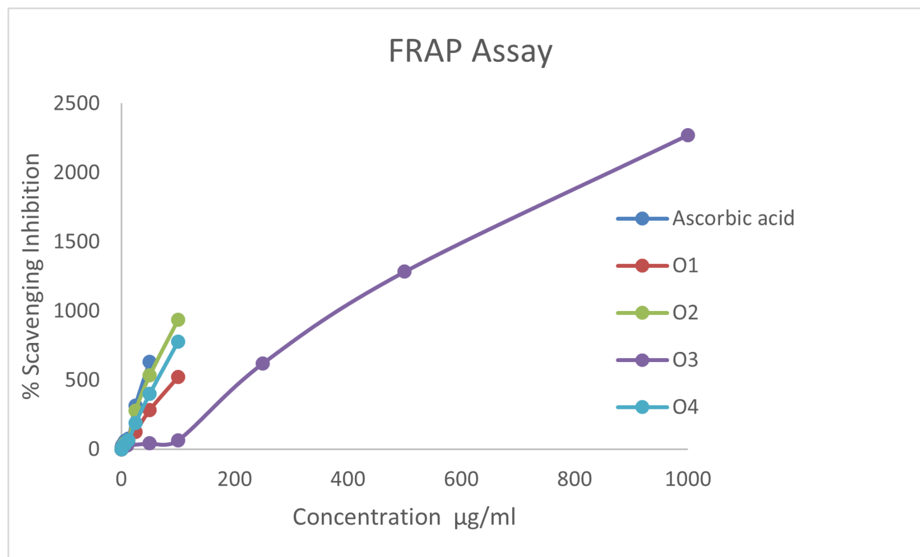

Figure 7: Total Antioxidant Assay-FRAP of Ascorbic acid, O1, O2, O3, and O4.

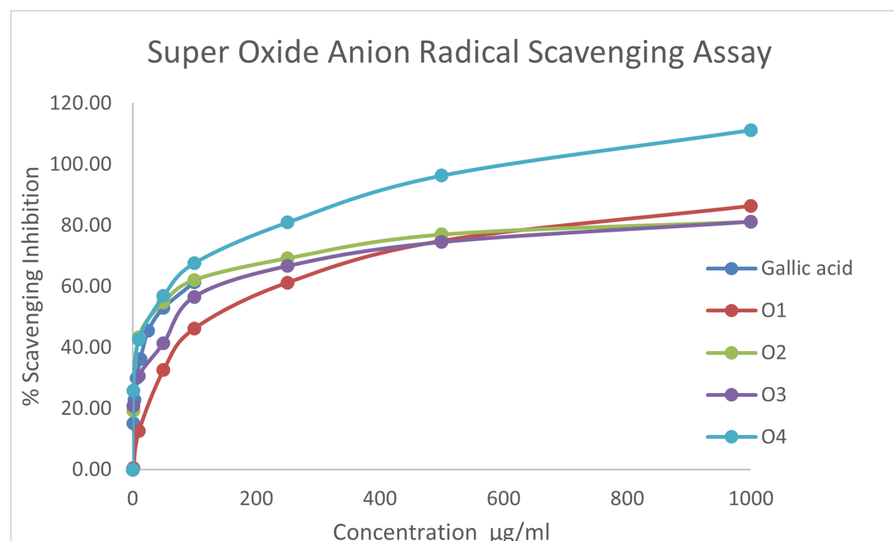

Figure 8: Super Oxide Anion Radical Scavenging Assay of Gallic acid, O1, O2, O3, and O4.

further validate the efficacy of this solvent solution for the effective recovery of bioactive components (Grover; Madhuri *et al*., 2021).

The phytochemical screening was meticulously performed to screen for the presence of flavonoids, tannins, phenols, and many other phytochemicals in the extracts. The proven antioxidant, anti-inflammatory, and antibacterial properties of these chemicals are of significant biological activity. *Acacia catechu* and *Allium cepa* were analyzed and found to possess tannins and flavonoids with a strong correlation to radical activity in tests such as ABTS and FRAP, which are recognized as radical scavengers. *Curcuma longa* contains phenols that increase its ability to scavenge hydroxyl radicals, as was observed in the CUPRAC experiment (Mankad *et al*., 2018).

The purity of extracts was evaluated by ash value, loss on drying, and solubility profiling to remove pollutants or excessive moisture, such procedures undermine stability and efficacy (Lakshmi *et al*., 2017).

The Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) are the most significant determinants of antioxidant activity because most molecules that stop free radicals and control oxidative stress are phenolics and flavonoids, respectively. Research has indicated that these compounds contain hydroxyl groups and conjugated structures, which neutralize reactive oxygen species and block oxidative damage. TPC, TFC, and antioxidant activity combine to form a robust association that enables their assessment as reliable indicators of the antioxidant capacity of a sample. This method allows for the evaluation of plant extracts, the standardization of antioxidant-containing products, and the approval of their use in food, pharmaceuticals, and cosmetics due to their protective and preservative properties (Pandey & Sharma, 2022).

The different extracts were assessed for free radical scavenging capacity by DPPH and ABTS radical scavenging assays with *Acacia catechu* extract showing significant free radical scavenging capacity. IC_{50} values represent each extract and their combinations in terms of the ability to mitigate oxidative stress. Since the combination of all three extracts enhanced activity in the superoxide anion scavenging assay, the synergistic interactions between the phytoconstituents were emphasized (Imam *et al*., 2011). Superoxide anion (O2-) is a Reactive Oxygen Species (ROS) produced in enzymatic reactions (e.g., NADPH oxidase, xanthine oxidase) and autoxidation of reduced molecules under conditions of mitochondrial respiration. Oxidative damage to cell components, such as lipids, proteins, and DNA, leads to the overproduction of O2- and oxidative stress. There is a rich history of Reactive Oxygen Species (ROS), where the formation of hydrogen peroxide, hydroxyl radicals, and peroxynitrite initiates the entire cascade. They cause structural and functional disruptions, lead to inflammation, apoptosis, and necrosis, impair cellular signaling, as well as trigger lipid peroxidation (Mouithys-Mickalad *et al*., 2024). The pharmacological effects of polyherbal drugs are often more potent than those of single herbal drugs, related mainly to the synergistic effects and the broad diversity of bioactive compounds. Polyherbal combinations are a blend of herbal drugs that contain multiple herbs, each contributing different antioxidants such as flavonoids and phenolic acids, which effectively combat superoxide anion and other Reactive Oxygen Species (ROS)(Wetchakul *et al*., 2019).

The hydroxy radical scavenging experiment further validated, as a powerful antioxidant, the capacity of the hydroxy component of *Curcuma longa* to neutralize reactive oxygen species known to be implicated in delayed physiological healing in the body. Through the CUPRAC and FRAP tests, subtracting the oxidative damage and taking cells suffering from repair, these extracts were able to achieve the process. Because the extract combination possesses enhanced hydroxy radical scavenging and overall antioxidant capability, the extract combination may provide or mitigate resistance to oxidative stress. The findings support the use of 'polyherbal therapies' in holistic healing, according to Ayurvedic principles (Porwal *et al*., 2024).

The antioxidant assays, in particular the DPPH, ABTS, and FRAP assays used to test the extract's ability to scavenge a broader range of Reactive Oxygen Species (ROS) or exhibit reducing power. The relatively lower activity seen in these assays suggests that the polyherbal combination might not all act equally efficiently against all kinds of Reactive Oxygen Species (ROS). Instead, its phytochemical composition may have more preference to attack superoxide radicals by structural or biochemical criteria (Rao *et al*., 2013).

CONCLUSION AND FUTURE PERSPECTIVES

In this study, a polyherbal combination of extract from *Curcuma longa, Acacia catechu*, and *Allium cepa* is examined for antioxidant properties. Further phytochemical studies observed bioactive substances, flavonoids, tannins, and phenols, whose outstanding medicinal potential is known. The polyherbal combination of extracts demonstrated superior radical scavenging activity in antioxidant assays using DPPH, ABTS, and FRAP compared to the component extracts. The Superoxide Anion Radical Scavenging Assay and other readouts verify the robust efficacy of the polyherbal extract and propose it as a specific antioxidant drug against disorders associated with excessive superoxide radical production. Consequently, synergistic interactions between phytochemicals, such as curcumin, catechin, and quercetin, are believed to target superoxide radicals specifically. The polyherbal extract possesses targeted efficacy against superoxide radicals. It represents a promising candidate for situations where superoxide-mediated oxidative damage is prevalent, such as chronic wounds with prolonged inflammation and various ROS-related diseases. Further studies could elucidate

the molecular basis for its selectivity and confirm its therapeutic effectiveness in relevant *in vivo* models.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

LOD: Loss on drying, **TPC:** Total phenolic content, **TFC:** Total flavonoid content, **IC_{50}:** Inhibition concentration, **DPPH:** 2,2-diphenyl-1-picrylhydrazyl, ABTS: (2,2′-azino-bis-(3-ethylb enzothiazoline-6-sulfonic acid), **CUPRAC:** Cupric reducing antioxidant capacity, **FRAP:** Ferric reducing antioxidant power, **ROS:** Reactive oxygen species.

REFERENCES

Azhar. (2007). *Polyherbal formulation concept for synergic action: a review*. http://www.sciencedirect.com/science/article/pii/S0160738315000444

Grover; Madhuri, Tapan Behl, Aayush Sehgal, Sukhbir Singh, Sharma; Neelam, Tarun Virmani, Mahesh Rachamalla, Abdullah Farasani, Sridevi Chigurupati, Amal M. Alsubayiel, Shatha Ghazi Felemban, Mohit Sanduja, & Simona Bungau. (2021). *In vitro* Phytochemical Screening, Cytotoxicity Studies of *Curcuma longa* Extracts with Isolation and Characterisation of their Isolated Compounds. *Molecules*, *26*, 1–17.

Imam, M. Z., Akter, S., Mazumder, E. H., & Rana, S. (2011). Antioxidant activities of different parts of *Musa sapientum* L. ssp. sylvestris fruit. *Journal of Applied Pharmaceutical Science*, *1*, 68–72. https://api.semanticscholar.org/CorpusID:55871975

Kambayashi, Y., Binh, N. T., W. Asakura, H., Hibino, Y., Hitomi, Y., Nakamura, H., & Ogino, K. (2009). Efficient Assay for Total Antioxidant Capacity in Human Plasma Using a 96-Well Microplate. *Journal of Clinical Biochemistry and Nutrition*, *44*(1), 46–51. https://doi.org/10.3164/jcbn.08-162

Kant, V., Jangir, B. L., Sharma, M., Kumar, V., & Joshi, V. G. (2021). Topical application of quercetin improves wound repair and regeneration in diabetic rats. *Immunopharmacology and Immunotoxicology*, *0*(0), 1–18. https://doi.org/10.1080/08923973.2021.1950758

Kumar Gupta, P., Vinayak Lokur, A., Praveen Kumar Gupta, C., & Rithu, B. (2019). Phytochemical screening and qualitative analysis of *Cymbopogon citratus. ~ 3338 ~* Journal of Pharmacognosy and Phytochemistry, *8*(4), 3338–3343.

Lakshmi, T., Ezhilarasan, D., Vijayaragavan, R., Bhullar, S. K., & Rajendran, R. (2017). *Acacia catechu* ethanolic bark extract induces apoptosis in human oral squamous carcinoma cells. *Journal of Advanced Pharmaceutical Technology & Research*, *8*(4), 143–149. https://doi.org/10.4103/japtr.JAPTR_73_17

Mankad, A., Pandya, D., & Joshi, B. (2018). Comparative study of Phytochemical Screening and Antibacterial Activity of *Curcuma longa* (L.) and *Curcuma aromatica* (Salib.). *Journal of Medicinal Plants Studies*, *6*, 145–148.

Mouithys-Mickalad, A., Etsè, K. S., Franck, T., Ceusters, J., Niesten, A., Graide, H., Deby-Dupont, G., Sandersen, C., & Serteyn, D. (2024). Free Radical Inhibition Using a Water-Soluble Curcumin Complex, NDS27: Mechanism Study Using EPR, Chemiluminescence, and Docking. *Antioxidants (Basel*, *Switzerland), 13*(1). https://doi.org/10.3390/antiox13010080

Nakhate, V. P., Akojwar, N. S., Sinha, S. K., Lomte, A. D., Dhobi, M., Itankar, P. R., & Prasad, S. K. (2023). Wound healing potential of *Acacia catechu* in streptozotocin-induced diabetic mice using *in vivo* and *in silico* approach. *Journal of Traditional and Complementary Medicine*, *13*(5), 489–499. https://doi.org/10.1016/j.jtcme.2023.05.001

Noda, Y., Anzai, K., Mori, A., Kohno, M., Shinmei, M., & Packer, L. (1997). Hydroxyl and superoxide anion radical scavenging activities of natural source antioxidants using the computerized JES-FR30 ESR spectrometer system. *Biochemistry and Molecular Biology International*, *42*(1), 35–44. https://doi.org/10.1080/15216549700202411

Pandey, L. K., & Sharma, K. R. (2022). Analysis of Phenolic and Flavonoid Content, α-Amylase Inhibitory and Free Radical Scavenging Activities of Some Medicinal Plants. *TheScientificWorldJournal*, *2022*, 4000707. https://doi.org/10.1155/2022/4000707

Pawar, H. (2014). Phytochemical Evaluation and Curcumin Content Determination of Turmeric Rhizomes Collected From Bhandara District of Maharashtra (India). *Medicinal Chemistry*, *4*(8), 588–591. https://doi.org/10.4172/2161-0444.1000198

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science : IJBS*, *4*(2), 89–96.

Porwal, M., Rastogi, V., Chandra, P., & Shukla, S. (2024). An Updated Review on the Role of Phytoconstituents in Modulating Signalling Pathways to Combat Skin Ageing: Nature's Own Weapons and Approaches. In *The Natural Products Journal* (Vol. 14, Issue 8, pp. 55–71). https://doi.org/http://dx.doi.org/10.2174/0122103155273789231122104742

Rahman, M. M., Islam, M. B., Biswas, M., & Khurshid Alam, A. H. M. (2015). *In vitro* antioxidant and free radical scavenging activity of different parts of *Tabebuia pallida* growing in Bangladesh. *BMC Research Notes*, *8*, 621. https://doi.org/10.1186/s13104-015-1618-6

Raina, N., Rani, R., & Gupta, M. (2021). *Angiogenesis: Aspects in wound healing* (pp. 77–90). https://doi.org/10.1016/B978-0-12-816196-8.00010-2

Rao, S. B., Jayanthi, M., Yogeetha, R., Ramakrishnaiah, H., & Nataraj, J. (2013). Free radical scavenging activity and reducing power of *Gnidia glauca* (fresen.) gilg. *Journal of Applied Pharmaceutical Science*, *3*(6), 203–207. https://doi.org/10.7324/JAPS.2013.3634

Rastogi, V., Porwal, M., Sikarwar, M. S., & Kumar, A. (2023). Phytoextract Loaded Nanoemulsions for Age-Defying Effects: A Review. *Journal of Young Pharmacists*, *15*(4), 604–615. https://doi.org/10.5530/jyp.2023.15.85

Rubio, C. P., Hernández-Ruiz, J., Martinez-Subiela, S., Tvarijonaviciute, A., & Ceron, J. J. (2016). Spectrophotometric assays for total antioxidant capacity (TAC) in dog serum: an update. *BMC Veterinary Research*, *12*(1), 166. https://doi.org/10.1186/s12917-016-0792-7

Salehi, B., Rodrigues, C. F., Peron, G., Dall, S., Sharifi-rad, J., Azmi, L., Shukla, I., Singh, U., Elissawy, A. M., Nasser, A., & Mishra, A. P. (2021). Curcumin nanoformulations for antimicrobial and wound healing purposes. *Phytotherapy Research*, *November 2020*, 1–13. https://doi.org/10.1002/ptr.6976

Saptarini, N. M., & Wardati, Y. (2020). Effect of Extraction Methods on Antioxidant Activity of Papery Skin Extracts and Fractions of Maja Cipanas Onion (*Allium cepa* L. var. ascalonicum). *The Scientific World Journal*, *2020*, 3280534. https://doi.org/10.1155/2020/3280534

Stohs, S. J., & Bagchi, D. (2015). Chemoprotective Properties of *Acacia catechu* Heartwood Extracts. *Phytotherapy Research*, *824*(November 2014), 818–824. https://doi.org/10.1002/ptr.5335

The Ayurvedic Pharmacopoeia of India, development and perspectives. (2017).

Tumilaar, S. G., Hardianto, A., Dohi, H., & Kurnia, D. (2023). A Comprehensive Review of Free Radicals, Oxidative Stress, and Antioxidants: Overview, Clinical Applications, Global Perspectives, Future Directions, and Mechanisms of Antioxidant Activity of Flavonoid Compounds. *Journal of Chemistry, 2024*. https://doi.org/10.1155/2024/5594386

V Rastogi, M Porwal, A. A. (n.d.). *An Overview of Nanotechnology-based Polyherbal Drug Delivery Systems for Wound Healing*.

Wetchakul, P., Goon, J. A., Adekoya, A. E., Olatunji, O. J., Ruangchuay, S., Jaisamut, P., Issuriya, A., Kunworarath, N., Limsuwan, S., & Chusri, S. (2019). Traditional tonifying polyherbal infusion, Jatu-Phala-Tiga, exerts antioxidant activities and extends lifespan of Caenorhabditis elegans. *BMC Complementary and Alternative Medicine*, *19*(1), 209. https://doi.org/10.1186/s12906-019-2626-1

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