

PHYTOCHEMICAL PROFILING AND ANTIOXIDANT ACTIVITY OF FORTIFIED
HERBAL MIXTURE

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ABSTRACT

Background: Ornamental plants are increasingly recognized for their aesthetic appeal as well as their potential pharmacological applications. This study investigates the phytochemical composition and antioxidant efficacy of a fortified formulation that combines *Sansevieria trifasciata* and *Tradescantia pallida*, enhanced with an extract from *Zingiber officinale*. **Methods:** The formulation underwent qualitative phytochemical screening to identify principal constituents, including alkaloids, glycosides, and proteins. The antioxidant potential was assessed using both DPPH and hydrogen peroxide (H_2O_2) radical scavenging assays. **Results:** Phytochemical analysis indicated a substantial presence of alkaloids, glycosides, and proteins across all components, with enhanced concentrations observed following fortification with ginger extract. The formulation demonstrated significant dose-dependent free radical scavenging activity, showing notable inhibition of both DPPH and H_2O_2 radicals. These effects can be attributed to the synergistic interactions among phenolic glycosides, nitrogenous alkaloids, and bioactive proteins sourced from the botanical constituents. **Conclusion:** The fortification of ornamental plants with *Zingiber officinale* enhances their antioxidant capacity, underscoring their potential application in natural therapeutic and cosmeceutical formulations. This study endorses the incorporation of traditionally aesthetic plants into evidence-based phytomedicine.

KEYWORD:- *Sansevieria trifasciata*, *Tradescantia pallida*, *Zingiber officinale*, phytochemicals, antioxidant, DPPH, hydrogen peroxide, fortification.

INTRODUCTION

For millennia, attractive plants have fascinated human senses and aesthetic preferences, resulting in the creation of numerous new cultivars. The primary objective of cultivating ornamental plants for commercial purposes is to develop innovative, visually striking cultivars characterized by enhanced flowering and other aesthetic features. Indoor plants are recognized as natural air purifiers due to their ability to improve air quality through processes such as filtration, precipitation, dilution, and absorption. Ornamental species such as *Sansevieria trifasciata* (Snake plant) and *Tradescantia pallida* (Purple heart) offer numerous advantages that extend beyond their visual appeal. The snake plant, acknowledged in NASA's Clean Air Study, is effective at eliminating common indoor air pollutants, including formaldehyde, benzene, and xylene, thereby significantly enhancing indoor air quality (Wolverton et al., 1989). Additionally, its Crassulacean Acid Metabolism (CAM) photosynthetic pathway facilitates oxygen production during the night, making it a suitable addition for bedrooms and confined spaces (Singh et al., 2020). In a similar vein, the purple heart plant, valued for its vibrant foliage, demonstrates pharmacological potential with

established antioxidant, antimicrobial, and anti-inflammatory properties attributed to its rich phenolic content (Santos et al., 2013). The innovative concept of enriching ornamental plants with bioactive agents, such as *Zingiber officinale* (ginger), presents a novel method to enhance their resilience and therapeutic value. Ginger is renowned for its potent anti-inflammatory, immunomodulatory, and antimicrobial properties, which may bolster plant defense mechanisms while augmenting the bioactive potential of plant extracts intended for topical use or air-purifying functions (Ali et al., 2008; Mao et al., 2019). This integrated approach not only elevates the functional and medicinal value of ornamental plants but also aligns with sustainable practices in urban horticulture and the development of phytopharmaceuticals.

MATERIALS AND METHOD

Collection of plant Material and Preparation

Fresh plant materials were sourced from the local market, ensuring their quality and authenticity. The collected leaves were thoroughly washed with distilled water to remove any impurities and then meticulously cleaned. A precise measurement of 10 grams each of

snake plant and purple heart leaves was taken before being crushed into small, manageable pieces. These crushed leaves were immersed in freshly extracted ginger juice, allowing the mixture to steep for five days, soaking up the vibrant flavors and properties of the ginger.

After the soaking period, the mixture was strained using a muslin cloth, effectively separating the leaves from the infused ginger juice. To maximize the extraction of beneficial compounds, the leaves were then submerged in the filtered infusion for an additional five days. Finally, the resultant extracts were gently concentrated over low heat, followed by careful drying to preserve their potent qualities.

Phytochemical analysis

1) Tests for carbohydrates

A. Molish's test (General test)

To 2-3 ml aqueous extract, add a few drops of alpha naphthol solution in an alcohol shake and add con. Sulphuric acid from the sides of the test tube. A violet ring is formed at the junction of the two liquids.

B. Tests for reducing sugars

- a) **Fehling's test:** Mix 1 ml of Fehling's A and Fehling's B solution and boil for 1 min. Add an equal volume of test solution to the test tube. Heat in boiling water bath for 5-10 min. First yellow, then brick red ppt is observed.
- b) **Benedict's test:** Mix equal volumes of Benedict's reagent and test solution in the test tube. Heat in boiling water bath for 5 min. The solution appears green-yellow or red depending on the amount of reducing sugars present in the test solution.

Tests for monosaccharides

- a) **Barfoed's test:** Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min boiling water bath and cool. Red ppt is observed.
- b) **Tests for pentose sugars:** Mix equal volume of the test solution and HCl. Heat and add a crystal of phloroglucinol red color appears.

C. Test for hexose sugars

- I) **Selwinoff's test:** Heat 3ml Selwinoff's reagent and 1ml test solution in a bearing water bath for 1-2 min. A red color is formed.
- II) **Tollen's phloro glucinol test for galactose:** Mix 2-5 ml con Hcl and 4 ml 0.5% phloro glucinol. Add 1-2 ml test solution. Heat. Yellow to red color appears.

D. Test for Non-reducing polysaccharide(Starch)

Iodine test: Mix 3 ml test solution and 3 drops of dilute iodine solution. A blue color appears, and it disappears on cooling.

2) Tests for proteins

- A. **Biuret test (General test):** To 3 ml test solution add 4% NaOH few drops of CuSO_4 Solution, violet or pink color appears.

Millions test: Mix 3 ml test solution with 5 ml reagent white. ppt warm ppt turns into pink color.

Xantho protein test: Mix 3 ml test solution with 1 ml con. Sulphuric acid. White PPT is formed. Boil PPT turns yellow. Add ammonium hydroxide. PPT turns orange.

3) Tests for amino acids

- A. **Ninhydrin test:** Heat 3 ml test solution and 3 drops of 5% Ninhydrin solution in the boiling Water bath for 10 min. The purple or bluish color appears.
- B. **Test for tyrosine:** Heat 3 ml of the test solution and add 3 drops of million's reagent. The solution shows a dark red color.
- C. **Test for cysteine:** To 5 ml test solution and a few drops of 40% NaOH and 10% lead Acetate solution. Boil. Black Ppt of lead sulfate is form

4) Tests for glycosides

A. Tests for cardiac glycosides

- a) **Baljet's test:** A thick section shows a yellow to orange color with sodium picrate.
- b) **Legal's test:** To aqueous or alcoholic extract, add 1 ml pyridine and 1 ml sodium nitroprusside. Pink to red color appears.
- c) **Keller killiani test:** To 2 ml extract add glacial acetic acid and one drop of 5% ferric chloride and con. Sulphuric acid. A reddish-brown color appears at the junction of the two liquid layers and the upper layer appears bluish-green.

B. Tests for anthra quinone glycosides

- a) **Borntrager's test:** To 3 ml extract add sulphuric acid. Boil and filtrate. To cold filtrate add equal vol benzene or chloroform. Shake well. Separate the organic solvent and add ammonia. The ammonical layer turns pink or red.
- b) **Modified Borntrager's test:** To 5 ml extract add 5 ml 5% ferric chloride and 5 ml dilute Hcl. Heat for 5 min in a boiling water bath. Cool and add benzene or any other organic solvent. Shake well. Separate organic layer. Add equal volume extract Ammonical layer turns pinkish red color.

C. Tests for saponin glycosides

- a) **Foam test:** Shake the drug extract or dry powder vigorously with water. Persistent foam observed.
- b) **Hemolytic test:** Add drug extract or dry powder to one drop of blood on the glass slide. Hemolytic zone appears

D. Tests for cyanogenetic glycosides

To dry drug powder or extract add 3% aqueous mercury nitrate solution. Metallic mercury forms.

E. Tests for coumarin glycosides: Alcoholic extract when made alkaline, shows blue or green fluorescence.

5) Tests for flavonoids

Shinoda test: To dry powder or extract, add 5 ml 95% ethanol, a few drops con. Hcl and 0.5 g magnesium

turnings. Pink colored observed. To a small amount of residue, add lead acetate solution. Yellow PPT is observed.

6) Tests For Steroids and Triterpenoids

- A. **Salkowski reaction:** To 2 ml extract, add 2 ml chloroform and 2 ml con. Sulphuric acid Shake well. The chloroform layer appears red and the acid layer shows greenish-yellow fluorescence.
- B. **Liebermann burchard reaction:** Mix 2 ml extract with chloroform. Add 1-2 ml acetic anhydride and add 2 drops of con. Sulphuric acid from the side of the test tube. First red, then blue and finally green color appears.
- C. **Sulphur powder test:** Add a small amount of sulfur powder to the test solution, it sinks at the bottom.

7) Tests for alkaloids

- A. **Dragen Dorff's reagent:** Alkaloids give reddish brown Ppt with this reagent. (mercuric iodide solution).
- B. **Mayer's reagent:** Alkaloids give cream color Ppt with Mayer's reagent. (Potassium mercuric iodide).
- C. **Wagner's reagent:** Alkaloids give reddish brown Ppt. (Iodine potassium iodide solution)
- D. **Hager's reagent:** Alkaloids give yellow Ppt (saturated solution of picric acid). Picrolonic acid Alkaloids give yellow Ppt.

8) Tests for phenolic compounds

Ferric chloride test: Treat the extract with ferric chloride solution, blue color appears if hydrolyzable tannins are present and green color appears if condensed tannins are present.


Test for chlorogenic acid: Treat the test solution with aq. Ammonia and exposure to air gradually green color is developed. Add Potassium dichromate: red Ppt. Add Bromine water. Discoloration of water

9) Fats and fixed oils

Saponification test: Add a few drops of 0.5N alcoholic potassium hydroxide to a small qty of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2 hrs. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils.

In vitro antioxidant methods

Table 1: Phytochemical Analysis of Prepared fortified mixture.

Name of the chemical test	procedure	Present/absent	Result/interference
Reducing sugar Fehlings's test:	Mix 1ml of fehling's a and fehling's b solution boil for 1min. Add an equal volume of test solution in a test tube. Heat in boiling water bath for 5-10min. First yellow then brick red ppt is observed.	Absent	

Hydrogen peroxide scavenging activity


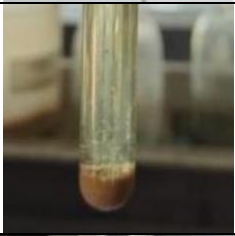






The ability of extracts to scavenge hydrogen peroxide was determined by little modification here the solution of hydrogen peroxide (100mM) was prepared instead of 40mM in phosphate buffer saline of (PH 7.4), at various concentrations of extract (10 -50 µg/ml) were added to the hydrogen peroxide solution (2 ml). The absorbance of hydrogen peroxide at 230 nm was determined after 10 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. A separate blank sample was used for background subtraction for each concentration. In the case of control takes the absorbance of hydrogen peroxide at 230 nm without sample extracts. Results are provided in the percentage inhibition activity was calculated from $[(A_0 - A_1)/A_0] \times 100$, where A0 is the absorbance of the control and A1 the absorbance of extract/standard taken as Ascorbic acid (10 - 50 µg/ml)

DPPH Radical Scavenging Assay

The antioxidant activity of the extracts is based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals. Plant extract (0.1 ml) was added to 3 ml of a .004% MeOH solution of DPPH. Water (0.1 ml) in place of the plant extract was used as a control. Absorbance at 517 nm was determined after 30 min, and the percent inhibition activity was calculated as $[(A_0 - A_1)/A_0] \times 100$, where A0 was the absorbance of the control, and A1 was the absorbance of the extract/standard.

RESULTS AND DISCUSSION

Generally, phytochemicals are considered research substances rather than necessary nutrients because there is currently insufficient evidence to support any potential health benefits. Major groups, including carotenoids and polyphenols, which include phenolic acids, flavonoids, stilbenes, or lignans, can be used to group phytochemicals under study. Based on their similar chemical structures, flavonoids can be further classified including anthocyanins, flavones, flavanones, isoflavones, and flavanols. In the present study, the zinger juice fortified mixture contains alkaloids, glycosides, and proteins, the remaining are shown as not present in the mixture (Table 1).

Test for Monosaccharides Pentose sugars	Mix an equal volume of the test solution and HCl. Heat and add a crystal of phloroglucinol, red color will appear.	Absent	
Test for Proteins Million's test	Mix 3ml test solution with 5ml reagent white.ppt warm ppt turns brick red or the ppt.	Present	
Test for amino acids: Ninhydrin test	Test 3ml test solution and 3 drops of 5% ninhydrin solution in a boiling water bath for 10 min. The purple or bluish color appears.	Absent	
Test for glycosides: Legal test	To aqueous or alcoholic extract, add 1ml pyridine and 1ml sodium nitroprusside. Pink or red color	Present	
Test for anthraquinone glycosides: Borntrager's test	To 3ml extract dil-sulphuric acid. Boil and filtrate. To cold filtrate add equal vol of benzene or chloroform. Shake well. Separate the organic solvent and add ammonia. The ammonical layer turns pink to red.	Absent	
Test for saponin glycosides: Foam test	Shake the drug extract or dry powder vigorously With water persistent foam is observed	Absent	
Test for steroids and triterpenoids : Salkowski reaction	To 2 ml extract, add 2 ml chloroform and 2 ml conc. Sulphuric acid shakes well. The chloroform layer appears red and the acid layers show greenish fluorescence.	Absent	
Test for alkaloids Hagers test	Alkaloids are given Yellow ppt[saturated solution of picric acid] picolinic acid alkaloids are given yellow ppt	Present	



Test for flavonoids: Lead acetate test	Take a small amount of plant extract and add lead acetate and it gives yellow ppt	Absent	
Test for fats and oils saponification test	Add a few drops of 0.5N alcoholic Potassium hydroxide to a small number of various extracts along with a drop of phenolphthalein separately and heat on a water bath for 1-2 hours. The formation of soap or partial neutralization of alkali indicates the presence of fixed oils.	Absent	

Table 2: Evaluation of DPPH radical scavenging activity of Fortified Mixture.

Concentration	Rutin	Fortified Mixture
	%Inhibition (DPPH)	
100mg/ml	32.76±1.23	22.28±1.39
200mg/ml	40.77±1.77	30.87±1.23
300mg/ml	51.23±1.46	49.68±1.45
400mg/ml	57.23±1.27	68.90±1.47
500mg/ml	62.30±1.42	78.93±1.81
IC50	316.20	289.46

Table 3: Evaluation of hydrogen peroxide scavenging activity of Fortified Mixture.

Concentration	Rutin	Fortified Mixture
	%Inhibition (H ₂ O ₂)	
100mg/ml	24.56 ±1.89	39.77±1.37
200mg/ml	31.25 ±1.44	43.48±1.49
300mg/ml	35.89±1.72	55.51±1.73
400mg/ml	43.67±1.23	62.95±1.34
500mg/ml	56.67 ±1.56	71.88±1.23
IC50	437.45	249.10

The qualitative phytochemical screening of the fortified formulation containing *Sansevieria trifasciata*, *Tradescantia pallida*, and *Zingiber officinale* has confirmed the presence of significant bioactive groups, specifically alkaloids, glycosides, and proteins, each contributing to the pharmacological profile of the formulation. Alkaloids represent a diverse class of nitrogenous compounds recognized for a broad spectrum of biological activities, including antioxidant, antimicrobial, and anti-inflammatory properties. The identification of alkaloids in all three botanical constituents aligns with previous research that has documented these compounds in *Sansevieria* spp. and *Zingiber officinale* (Sivakumar et al., 2010; Ali et al., 2008). These secondary metabolites primarily exert their antioxidant effects through radical scavenging and metal-chelating mechanisms, thereby mitigating oxidative stress-induced damage within biological systems (Kurek-Górecka et al., 2020). Glycosides, particularly those classified as phenolic and flavonoid glycosides, are abundantly present in *Tradescantia pallida* and *Zingiber officinale*, and their inclusion was confirmed in the

fortified formulation. Glycosides are essential for cellular protection due to their capability to stabilize reactive oxygen species (ROS) and modulate enzyme systems implicated in detoxification and inflammation (Santos et al., 2013; Mao et al., 2019). Their antioxidant activity is largely attributed to hydroxyl groups attached to aromatic rings, which effectively donate electrons or hydrogen atoms to neutralize free radicals such as DPPH and hydrogen peroxide. This is consistent with the pronounced scavenging activity demonstrated in both DPPH and H₂O₂ assays. Although proteins are not typically emphasized in antioxidant research, they have recently garnered attention for their potential in radical quenching, particularly enzymatic antioxidants like peroxidases and superoxide dismutase-like proteins present in plant tissues. The identification of proteins in this study indicates a possible contribution to the overall redox balance of the formulation. Furthermore, certain low molecular weight peptides derived from plant proteins are recognized for their direct free radical-scavenging capabilities (Udenigwe & Aluko, 2012). The synergistic combination of alkaloids, glycosides, and

proteins enhances the biological potential of the formulation, thereby reinforcing its antioxidant properties, as evidenced by the free radical assays. The collective effects of these biomolecules sourced from ornamental and medicinal plants provide a scientific foundation for the fortification strategy utilizing *Zingiber officinale*, consequently positioning the formulation as a promising candidate for phytotherapeutic and cosmeceutical applications.

CONCLUSIONS

The present study demonstrates that fortification of ornamental plants *Sansevieria trifasciata* and *Tradescantia pallida* with *Zingiber officinale* significantly enhances the phytochemical profile and antioxidant potential of the resulting formulation. Phytochemical screening confirmed the presence of vital secondary metabolites, particularly alkaloids, glycosides, and proteins, which are known to contribute synergistically to antioxidant defense mechanisms. The free radical scavenging assays using DPPH and hydrogen peroxide provided compelling evidence of the formulation's ability to neutralize oxidative stress, likely mediated through electron donation, hydrogen transfer, and metal ion chelation. The integration of bioactive-rich ginger into a formulation based on underutilized but pharmacologically promising ornamental plants not only improves therapeutic efficacy but also offers an innovative approach to value addition in both medicinal and cosmetic formulations. These findings suggest that the fortified combination holds potential for further development as a natural antioxidant supplement or cosmeceutical agent, supporting future investigations into its pharmacological applications and safety in clinical models.

ABBREVIATIONS

DNA: Deoxyribonucleic acid
 DPPH: 2,2-diphenyl-1-picrylhydrazyl
 H₂O₂: Hydrogen peroxide
 MeOH: Methanol
 NaOH: Sodium Hydroxide

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