



CODEN [USA]: IAJPB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**

SJIF Impact Factor: 7.187

<https://zenodo.org/records/11210370><https://www.iajps.com/volumes/volume11-may-2024/14-issue-05-may-24/>Available online at: <http://www.iajps.com>

Research Article

**PHYTOCHEMICAL SCREENING AND EVALUATION OF ANTI-
OXIDANT ACTIVITY OF *DRACAENA TRIFASCIATA* AND
ZAMIOCULCAS ZAMIIFOLIA: IN-VITRO STUDY**Sai Deepika L, Hyma A, Bhavani K, Sailaja B, Deepthi N, Asif Sk, Udaya J, Jagadeesh P
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Abstract:

Dracaena trifasciata (Snake plant) and *Zamioculcas zamiifolia* (ZZ plant) are widely used ornamental plants. This research aimed to investigate the phytochemistry and in vitro antioxidant activity of *D.trifasciata* and *Z. zamiifolia* leaves. Freshly prepared juice of *D. trifasciata* contains flavonoids, phenol, glycosides, terpenoids, monosaccharides, and amino acids, and *Z. zamiifolia* contains only carbohydrates. In vitro antioxidant studies have shown potential effects in scavenging DPPH and hydrogen peroxide in a concentration-dependent manner. These activities may be due to phytochemicals present in the selected ornamental plants.

Keywords: *Dracaena trifasciata*, *Zamioculcas zamiifolia*, ornamental plants, DPPH, Hydrogen Peroxide

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Please cite this article in press Swathi Putta et al., *Phytochemical Screening And Evaluation Of Anti-oxidant Activity Of Dracaena Trifasciata And Zamioculcas Zamiifolia: In-Vitro Study.*, *Indo Am. J. P. Sci*, 2024; 11 (05).

INTRODUCTION:

Ornamental plants are grown for decorative purposes in gardens and landscapes. These plants are used for design projects, houseplants, cut flowers, and displays, attracting attention due to their attractive colors, beautiful designs, and fascinating appearance [1]. They can be displayed both indoors and outdoors as decoration, as they have vibrant colors and add life to any environment. The cultivation of ornamental plants is called floriculture, which forms the main branch of horticulture. Ornamental plants are grown to exhibit aesthetic features including flowers, leaves, fragrance, overall leaf structure, fruit, stem and bark, and aesthetic form [2]. The most popular ornamental plants used are *Dracaena trifasciata* and *Zamioculcas zamiifolia*.

Dracaena trifasciata (snake plant) is a species of flowering plant in the Asparagaceae family, native to tropical West Africa from Nigeria east to the Congo. It is most commonly known as a snake plant [3]. Snake plants are popular ornamental plants that treat diabetes and hemorrhoids, inhibit the growth of cancer cells, act against poisonous snakes and insect textile raw materials, and clean 107 kinds of pollutants in the air [4]. The plant exchanges oxygen and carbon dioxide using the crassulacean acid metabolism process, which allows it to withstand drought. *D. trifasciata* has traditionally been used for skin diseases, fungal diseases, ear infections, earaches, toothaches, hemorrhoids, ulcers, intestinal worms, stomach problems, diarrhea, and diabetes, promotes mental health and is effective against allergies [5]. They are clinically proven for their detoxification effect by removing carbon dioxide and releasing oxygen at night, helping to ensure a better quality of life and a healthy environment, and helping to absorb benzene, xylene, and formaldehyde released by cooking, smoking, cosmetics, and paint, etc. [6]. *Zamioculcas zamiifolia* (ZZ plant) belongs to the Araceae family. It is a stemless tropical herbaceous monocot native to East Africa, from southern Kenya to northeastern South Africa, growing on stony soil or tropical moist forest floor. It is cultivated as a medicinal-ornamental plant, mostly for its attractive glossy foliage and some pharmaceutical metabolites [7]. In addition, the ZZ plant has the potential to reduce the concentration of polluting gases such as benzene, ethylbenzene, xylene, and toluene from contaminated indoor air. It has the properties of traditional medicine due to the presence of steroids, triterpenoids, flavonoids, and polyphenols [8]. The extract is also a source of antioxidants and the juice is used in Tanzania to treat earaches. Locals also use the whole plant to treat inflammatory conditions. Therefore, due to the lack of

scientific reports on the selected indoor ornamental plants such as *Dracaena trifasciata* and *Zamioculcas zamiifolia*, the present investigation was carried out to identify the potential phytochemicals and actions toward scavenging the free radicals using several *in vitro* methods.

MATERIALS AND METHODS:**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 boiling 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, 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. 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. Dragendorff'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:

Hydrogen Peroxide Scavenging Activity [9]

Hydrogen peroxide radical scavenging assay

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 s

the absorbance of extract/standard taken as Ascorbic acid (10 - 50 µg/ml) (Gülçin, 2005).







Dpph Radical Scavenging Assay [10]








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 A_0 was the absorbance of the control, and A_1 was the absorbance of the extract/standard.

RESULTS & DISCUSSION:

Phytochemicals are non-nutritive plant chemicals that have either defensive or protective properties against disease. They are non-essential nutrients and are mainly produced by plants to provide them with protection [11]. Dietary intake of phytochemicals may promote health benefits, and protect against chronic degenerative disorders such as cancer and cardiovascular and neurodegenerative diseases. Most foods, such as whole grains, beans, fruits, vegetables, and herbs, contain phytochemicals. These phytochemicals, either alone and/or in combination, have enormous therapeutic potential in the treatment of various diseases [12].

Table 1: Preliminary Phytochemical analysis of *Dracaena Trifasciata*

Name of the chemical test	Observation	Present/absent	Result/interference
TEST FOR PROTEINS: A. Millons test	No change in the color	Absent	
TEST FOR GLYCOSIDES A.Legals test	The pink or red color is observed	Present	
TEST FOR NON-REDUCING SUGARS A.iodine test	Blue colour is not observed	Absent	
TEST FOR SAPONIN GLYCOSIDES A.Foam test	Foam is observed	Present	
TEST FOR ALAKLOIDS A.Hager's reagent	Turns into yellow colour ,yellow colour ppt is not observed	Absent	
TEST FOR PHENOLIC COMPOUNDS: A.Ferric chloride test:	Green colour is observed	Present	

<p>TEST FOR CARBOHYDRATES: A.molisch's test</p>	<p>The violet colour ring is not formed at the junction of two liquids.</p>	<p>Absent</p> 
<p>TEST FOR REDUCING SUGARS A.Fehling's test</p>	<p>The brick red ppt is not observed.</p>	<p>Absent</p> 
<p>TEST FOR MONOSACCHARIDES A.Tests for pentose sugars</p>	<p>Brick brick-red color is observed</p>	<p>Present</p> 
<p>TEST FOR ANTHRAQUINONE GLYCOSIDES A.Borntrager's test:</p>	<p>Phase separation is observed. The ammonical layer doesn't turn to a pink color</p>	<p>Absent</p> 
<p>TEST FOR AMINOACIDS A.ninhydrin test</p>	<p>The blue color is observed</p>	<p>present</p> 
<p>TEST FOR FLAVONOIDS A.Lead acetate test</p>	<p>Formation of yellow ppt is observed.</p>	<p>Present</p> 
<p>TEST FOR STEROIDS & TRITERPENOIDS A.Salkowski reaction</p>	<p>The organic layer and aqueous layer get separated. chloroform layer appears red and the acid layer shows green</p>	<p>Present</p> 










	fluorescence is observed.		
FATS AND FIXED OILS: A. Saponification test	The formation of soap is not observed.	Absent	

Table 2: Phytochemical Analysis of *Zamioculcas zamiifolia*

Name of the chemical test	Observation	Present/absent	Result/interference
Carbohydrates Fehling's test	A dark blue color was observed	Positive	
Carbohydrates (reducing sugars) Benedict's test	A greenish-yellow colour was observed	Positive	
Amino acids Ninhydrin test	Purple/bluish colour was not observed	Negative	
Anthra quinone glycosides Borntrager's test	The ammonical layer does not show pink/red	Negative	
Saponin glycosides Foam test	Foam was not observed	Negative	

Proteins Millions test	Brick red precipitate was not observed	Negative	
Steroids and triterpenoid Salkowski test	Greenish-yellow fluorescence was not observed	Negative	
Fats and fixed oils Saponification test	White foam was not observed	Negative	

Currently, there has been a huge increase in natural product research in the search for natural antioxidants as an alternative to synthetic drugs. Due to its low side effects compared to synthetic drugs [13]. Natural antioxidants show good potential to act as therapeutic agents in their mechanism of elimination of radical chain reactions in biological systems. Growing experimental evidence suggests that their plant products influence many cellular events in terms of their free radical scavenging activity. The antioxidant vitamin C has been found in animals and plants. It is not synthesized biologically and most of it can be obtained from food. Ascorbic acid is stored in a reduced form in combination with glutathione and supports disulfide isomerase and glutaredoxin proteins. It acts as a reducing agent like hydrogen peroxide to neutralize free radicals. Free radicals are divided into oxygen and nitrogen free radicals according to their performance [14]. Excessive free radical production can be caused by uncontrolled stress caused by an imbalance between the body's natural defenses in the formation of stress-induced oxidative stress. Radicals are involved in cellular components that cause injury or death [15]. The production of all kinds of free radicals is stored in

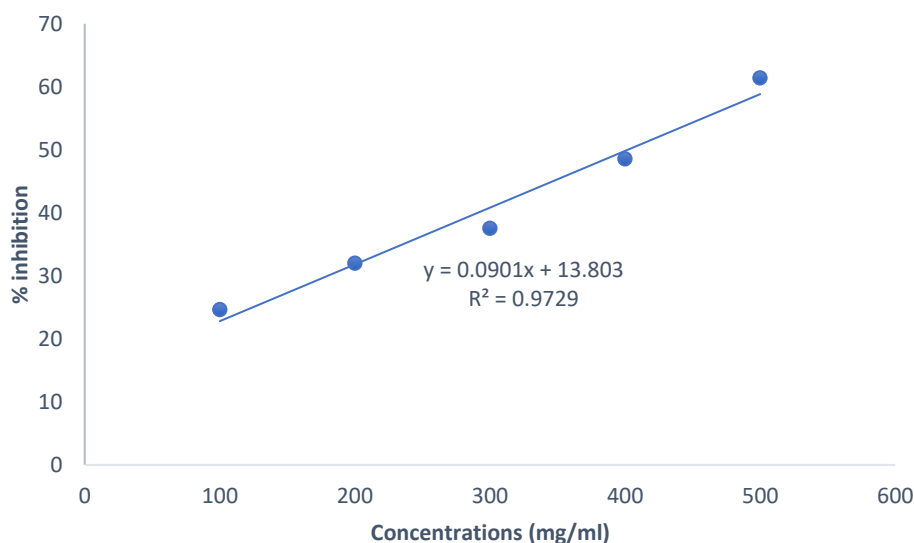
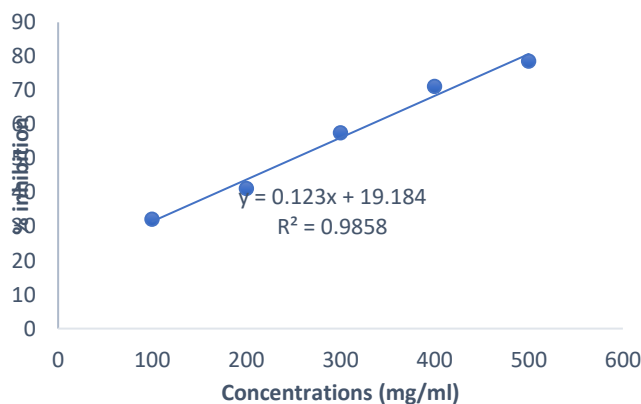
normal cells, and overproduction can now be considered the main cause of many diseases. Oxidative stress is considered a major factor in many chronic conditions such as diabetes, tumors, neuropathy, gastric ulcers, and others [15].

Therefore, this study was designed to evaluate the effectiveness of selected leaf extract on stress using in-vitro scavenging activity of hydrogen peroxide and DPPH radicals.

Hydrogen peroxide is produced in vivo in an impaired reaction catalyzed by the enzyme superoxide dismutase (SOD). It is not a free radical and can even cause cell damage at low levels, but at high levels, it inactivates cellular enzymes that produce enzymes such as glyceraldehyde-3-phosphate dehydrogenase [16]. It can easily penetrate the membrane of biological systems, but it does not directly affect DNA, but it can damage DNA by producing hydroxyl radical ions (OH⁻) in the presence of transition metal ions [17]. This study shows that *Dracaena trifasciata* and *Zamioculcas zamiifolia* show a greater inhibition effect by hydrogen peroxide in a concentration-dependent manner (Table 3 and Figures 1 and 2).

Table 3: Evaluation of hydrogen peroxide scavenging activity of *Dracaena trifasciata* and *Zamioculcas zamiifolia*

CONCENTRATION	<i>Dracaena trifasciata</i>	<i>Zamioculcas zamiifolia</i>
	%Inhibition (H₂O₂)	
100mg/ml	24.64±1.54	23.24±2.11
200mg/ml	31.99 ± 2.10	37.52±2.43
300mg/ml	37.51 ± 2.09	53.66±1.53
400mg/ml	48.53 ± 1.56	67.34±1.76
500mg/ml	74.81±1.89	80.33±1.98
IC50	402.74	250.53

Figure 1: % Inhibition of Hydrogen peroxide activity of *Dracaena trifasciata*Figure 2: %Inhibition of Hydrogen peroxide activity of *Zamioculcas zamiifolia*

DPPH is a known scavenger of other radicals. The *in vitro* antioxidant activity of *Dracaena trifasciata* and *Zamioculcas zamiifolia* may be due to their hydrogen-donating or electron-donating ability. Phytochemicals such as carbohydrates and reducing sugars present in *Zamioculcas zamiifolia* must be responsible for scavenging hydrogen peroxide and DPPH radicals in a concentration-dependent manner. The reaction of DPPH with an antioxidant or reducing complex produces the corresponding hydrazine DPPH, which can be followed by a color change from purple

to yellow [18]. The selected leaves showed that their effect on DPPH is probably due to their hydrogen-yielding powder capacity (Table 4 and Figures 3 and 4)

Table 4: Evaluation of DPPH radical scavenging activity of *Dracaena trifasciata* and *Zamioculcas zamiifolia*

CONCENTRATION	<i>Dracaena trifasciata</i>	<i>Zamioculcas zamiifolia</i>
	%Inhibition (DPPH)	
100mg/ml	42.50±2.32	31.91±1.67
200mg/ml	46.00±1.98	41.65±1.98
300mg/ml	60.77±1.67	52.64±2.45
400mg/ml	70.41±2.44	60.99±2.15
500mg/ml	74.81±2.89	69.99±1.56
IC50	200.4	135.25

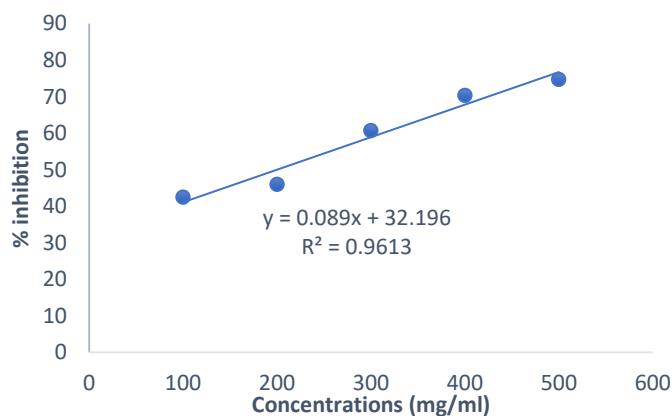


Figure 3: % Inhibition of DPPH radical activity of *Dracaena trifasciata*

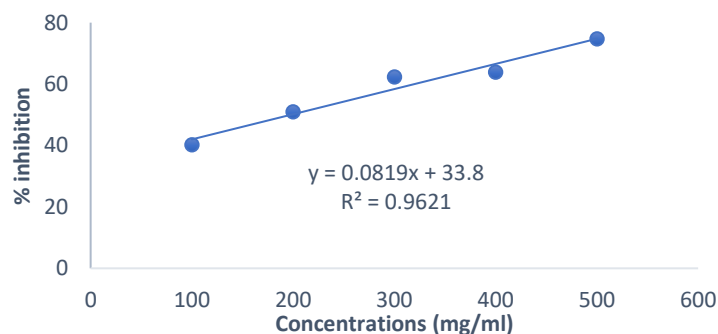


Figure 4: Percentage Inhibition of DPPH Radical with *Zamioculcas zamiifolia*

CONCLUSIONS:

The phytochemicals in *Dracaena trifasciata* leaves such as carbohydrates, amino acids, glycosides, saponins, steroids, and triterpenoids, flavonoids, and phenols *Dracaena trifasciata*; as carbohydrates, reducing sugars present in *Zamioculcas zamiifolia* might be responsible for scavenging DPPH and hydrogen peroxide in a concentration-dependent manner. The *in-vitro* anti-oxidant activity of *Dracaena trifasciata* & *Zamioculcas zamiifolia* might have

electron-donating and hydrogen-donating capabilities. Further, the study needs extensive research on the effect of selected ornamental plants on various models of antioxidant assays and *in vivo* correlation.

ABBREVIATIONS:

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

NaOH: Sodium Hydroxide

ACKNOWLEDGEMENTS: We express our gratitude to the management of Raghu College of Pharmacy, Visakhapatnam for providing us with the necessary facilities to carry out our research.

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