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(RESEARCH ARTICLE)

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Firsthand data acquisition for hypertension, its amelioration by extract of *Bougainvillea spectabilis* and formulation of nanoparticles

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Abstract

Hypertension is a widespread cardiovascular condition characterized by persistently elevated blood pressure, leading to serious health complications such as heart disease, stroke, and renal failure. This study investigates the antihypertensive properties of *Bougainvillea spectabilis* extract, focusing on its potential mechanisms of action and therapeutic efficacy. Using a controlled experimental design, hypertensive rats were treated with varying doses of *Bougainvillea spectabilis* extract. Blood pressure measurements were taken before and after treatment using a non-invasive tail-cuff method. Phytochemical analysis of the extract revealed a rich composition of bioactive compounds, including flavonoids, alkaloids, and tannins, known for their cardioprotective and vasodilatory effects. Results demonstrated a significant reduction in both systolic and diastolic blood pressure in treated subjects compared to the control group. Additionally, improvements in endothelial function were observed, indicated by increased nitric oxide levels and decreased oxidative stress markers. Histological analysis of vascular tissues showed enhanced integrity and reduced inflammation. These findings suggest that *Bougainvillea spectabilis* extract exerts a dual action: promoting vasodilation and improving endothelial health. The results support the potential of *Bougainvillea spectabilis* as a natural antihypertensive agent, highlighting the need for further research to elucidate its mechanisms and evaluate its clinical applicability in managing hypertension.

Keywords: Hypertension; *Bougainvillea spectabilis;* Antioxidant; Antioxidant; Phytochemicals; Antimicrobial Activity

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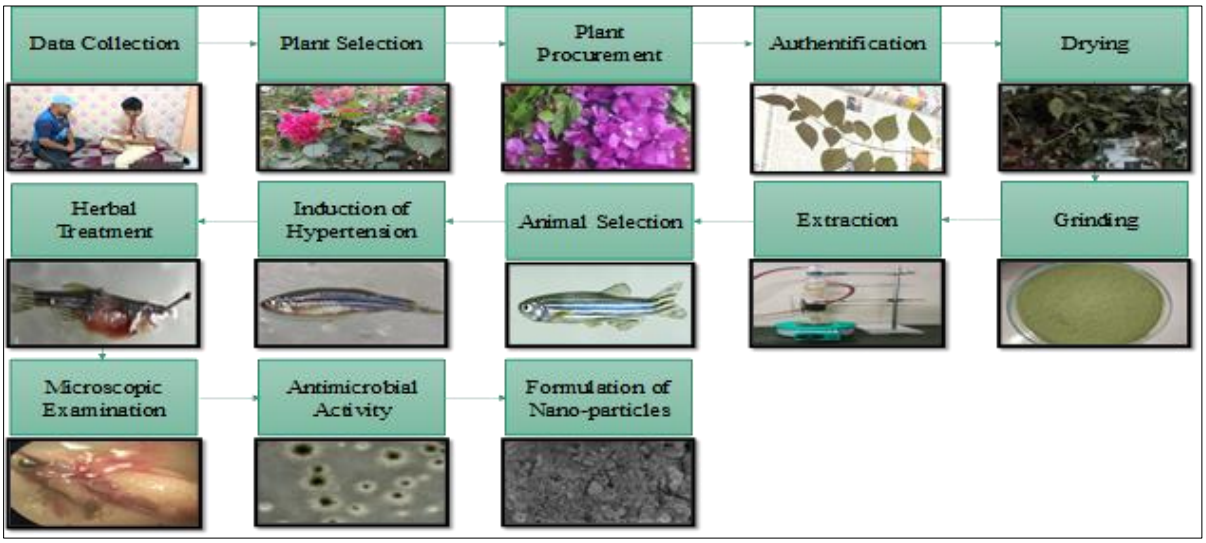
Graphical Abstract



1. Introduction

Hypertension is a chronic medical condition characterized by persistently elevated blood pressure, defined as systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 80 mmHg. It is a significant global health issue, affecting approximately 1.4 billion people worldwide, with prevalence rates continuously rising due to factors such as sedentary lifestyles, poor dietary choices, and increased stress levels. Untreated hypertension can lead to serious complications, including myocardial infarction, stroke, chronic kidney disease, and heart failure. The economic burden on healthcare systems is substantial, highlighting the urgent need for effective management strategies [1]. Conventional treatment for hypertension primarily involves the use of antihypertensive medications, including diuretics, beta-blockers, ACE inhibitors, and calcium channel blockers. While these medications are effective for many, they often come with side effects and do not work for all patients. Furthermore, a significant number of individuals remain untreated or are non-compliant with medication regimens, leading to uncontrolled hypertension. This underscores the need for complementary and alternative therapies that can be safely integrated into existing treatment protocols [2]. *Bougainvillea spectabilis*, commonly known as the flame tree, is a species native to tropical regions and widely recognized for its striking red flowers. Traditionally, various parts of the plant—including leaves, flowers, and pods—have been used in folk medicine for their purported health benefits, including the treatment of respiratory conditions, inflammation, and infections [3].

Figure 1 *Bougainvillea spectabilis* Plant

Recent phytochemical studies have identified a variety of bioactive compounds within *Bougainvillea spectabilis*, such as flavonoids, alkaloids, tannins, and phenolic acids. These compounds are known to exhibit antioxidant properties, which help mitigate oxidative stress—a contributing factor in the pathophysiology of hypertension. Research suggests that the

antihypertensive effects of *Bougainvillea spectabilis* may be mediated through multiple mechanisms. These include the relaxation of vascular smooth muscle, improvement of endothelial function, and reduction of inflammation [4]. Flavonoids are believed to enhance nitric oxide bioavailability, promoting vasodilation and improving blood flow. Additionally, the anti-inflammatory properties of the extract may counteract the vascular inflammation often observed in hypertensive patients. This study aims to explore the efficacy of *Bougainvillea spectabilis* extract in the management of hypertension by evaluating its impact on blood pressure and underlying mechanisms [5]. Through a series of experiments, we seek to elucidate how this natural extract can potentially serve as a complementary treatment for hypertension, offering a safer alternative for patients seeking to manage their condition holistically. By understanding the pharmacological potential of *Bougainvillea spectabilis*, we hope to contribute to the development of more effective strategies for hypertension management, ultimately improving patient outcomes and quality of life [6].

2. Material and methods

Following steps have been followed in reference to find a particular problem for specific population and its solution in terms of treatment or prevention by herbal formulation.

- **Firsthand Data Acquisition:** It was observed that the hypertension is one of the common spreading disorders. The following procedure was performed to collect the data for further research study [7].
- **Designing the questionnaire:** Two types of questionnaires have been designed out of which one was for all the subjects, and another was only for hypertensive patients [8].
- **Sampling:** The subjects was selected from different geographical regions all over India. They have been categorised into hypertensive patients and subjects with normal blood pressure [9].
- **Conducting Interviews:** The questionnaire was given to all selected subjects and one on one interview have been conducted [10].

2.1. Data Analysis

The collected data was analysed not only as quantitatively but also qualitatively too. The wide range of subjects from different regions were undergone the interview of this research study. The data collected by the questionnaire further evaluated to get a specific conclusion about number of people those who are suffering from hypertension [11].

2.2. Plant Procurement and Extraction

The leaves of *Bougainvillea spectabilis* was collected from local residential areas of Aurangabad. The herbarium was made and authentification has been done by Dept. of botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Leaves were dried in shady area by avoiding direct exposure to sunlight to avoid catalytic reactions by UV light. Dried leaves were than grinded to get course powder. The total of 1 kg coarse powder was extracted in 2.5 L methanol by Soxhlet apparatus for 72 hours. The extracted sample was collected and dried in atmospheric air in shady area to evaporate excessive solvent [12].

2.3. Preliminary Phytochemical Screening

Phytochemical screening was conducted to identify the presence of various bioactive compounds in the *Bougainvillea spectabilis* extracts. The tests were carried out following standard procedures for detecting the presence of alkaloids, flavonoids, saponins, tannins, glycosides, terpenoids, phenols, and others [13].

Table 1 Tests Performed for Preliminary Phytochemical Screening of Plant Extract [14]

Sr. No.	Phytochemicals	Tests Performed	Procedure
1	Alkaloids	Mayer's Reagent Test	2 mL of Mayer's Reagent was Added to 5 mL of Plant Extract.
		Dragendroff's Reagent Test	1 mL of Mayer's Reagent was Added to 2 mL of Plant Extract.
2	Flavonoids	Shinoda Test (Mg^{2+} Ribbon Test)	Extract was Dissolved in 95% Ethanol. To this, Small Piece of Mg Foil Metal was Added. Then 3 Drops of Conc. HCl was Added.

3	Tannins	Ferric Chloride Test	1 mL of Crystal Solution of Extract was Taken in a Test Tube. Few Drops of 1%$FeCl_3$ was Added.
4	Saponins	Froth Test	Extract was added in a Water in Test Tube. It was Shaken for 5 Minutes.
5	Terpinoids	Salkowski Test	5 mL of Extract was Mixed in 2 mL $CHCl_3$. Then 2 mL of H_2SO_4 was Added to Form a Layer
6	Glycosides	Killer-Killiani Test	1 mL of Glacial Acetic Acid was Added to 1 mL of Plant Extract and Cooled. After Cooling, 2 Drops of $FeCl_3$ was Added Followed by Careful Addition of Conc. H_2SO_4 along the Walls of the Test Tube.
7	Phenols	Ferric Chloride Test	Few Drops of 1% FeCl3 was Added in 2 mL of Plant Extract
8	Carbohydrates	Molisch's Test	3 Drops of Molisch's Reagent were Added in 2 mL of Extract.

2.4. Animal Study

The selected animal for the study was the zebrafish (Danio rerio). The zebrafish were acclimatized to their environment by keeping them in an aquarium for 30 days. They were then divided into six experimental groups, with five fish in each group: control, hypertension-induced, positive control, test-1, test-2, and test-3. The control group was not treated with any substances. The hypertension-induced, positive control, and test groups were subjected to chemical induction of hypertension using L-NAME (Nω-Nitro-L-arginine methyl ester), a nitric oxide synthase inhibitor. L-NAME was dissolved in the aquarium water, and the fish were allowed to swim in it for 72 hours. After this induction period, the fish were transferred to normal tap water for recovery [15]. Based on preliminary studies, the *Bougainvillea spectabilis* extract was administered at doses of 25 mg/L, 50 mg/L, and 100 mg/L per kg body weight to test-1, test-2, and test-3 groups, respectively. These doses were administered twice daily (every 12 hours) for a total of 7 days. The positive control group received the standard antihypertensive drug, captopril, with the same dosing schedule. The behaviours of all the groups were monitored and compared to those of the control and hypertension-induced groups. At the end of the experiment, the fish were euthanized, and their hearts were dissected for histopathological analysis. Hematoxylin and eosin stains were used for microscopic examination of heart tissues [16].

2.5. Formulation and Development of Extract

The concentrated extract of *Bougainvillea spectabilis* (20 mL) was added to a solution of Silver Nitrate ($AgNO_3$). The mixture was stirred at room temperature, and the colour change from yellow to dark brown indicated the formation of nanoparticles. The reaction was allowed to proceed for 4 hours, after which the nanoparticles were separated from the solution by centrifugation at 5,000 rpm. The supernatant was discarded, and the nanoparticles were washed several times with distilled water to remove any unreacted plant extract or byproducts. After washing, the nanoparticles were redispersed in ethanol. To confirm the formation of nanoparticles, the absorbance was measured using UV-Visible spectroscopy. To prevent aggregation or instability of the nanoparticles, polyvinyl alcohol (PVA) was added, and the formulation was stored in a cool, dry place [17].

3. Results

3.1. Firsthand Data Acquisition

The collected data demonstrate that the hypertension is most common in the age above 45 years [7,10,11].

3.2. Plant Procurement and Extraction

The yield of dried plant extract was found to be 13.8 g [12].

3.3. Preliminary Phytochemical Screening

The results from the preliminary phytochemical screening of the *Bougainvillea spectabilis* leaf and flower extracts revealed the presence of several bioactive compounds, as detailed below [13].

Table 2 Observations of Preliminary Phytochemical Analysis [14]

Sr. No.	Phytochemicals	Tests Performed	Observation	Inference
1	Alkaloids	Mayer's Reagent Test	White or Creamy Precipitate.	Positive
		Dragendroff's Reagent Test	Orange or Orange-red Precipitate.	Positive
2	Flavonoids	Shinoda Test (Mg^{2+} Ribbon Test)	Intense Cherry Red Colour.	Positive
3	Tannins	Ferric Chloride Test	An intense Reddish-Brown Colour.	Positive
4	Saponins	Froth Test	1 cm Layer of Foam.	Positive
5	Terpinoids	Salkowski Test	Reddish-Brown Colour.	Positive
6	Glycosides	Killer-Killiani Test	Reddish-Brown Layer Formed & Then Turned into Bluish Green After Allowed to Stand.	Positive
7	Phenols	Ferric Chloride Test	Bluish Green Colour.	Positive
8	Carbohydrates	Molisch's Test	Purple-Violet Colour.	Positive

- **Alkaloids:** The presence of alkaloids was confirmed in both leaf and flower extracts through the Dragendorff's reagent test, which produces an orange-brown precipitate when alkaloids are present [13].
- **Flavonoids:** Both leaf and flower extracts tested positive for flavonoids, as indicated by a red colour formation during the Shinoda test, which is characteristic of flavonoids [13,14].
- **Tannins:** Tannins were present in both the leaf and flower extracts. The formation of a blue-black coloration upon treatment with ferric chloride suggests the presence of hydrolysable tannins [13].
- **Saponins:** The frothing test confirmed the presence of saponins in both extracts. Saponins produce a stable foam when shaken with water [14].
- **Terpenoids:** The Salkowski test for terpenoids was positive, with the formation of a red layer at the interface, indicating the presence of terpenes [12].
- **Glycosides:** Glycosides were detected using the Keller-Killiani test, which resulted in the formation of a reddish-brown ring at the interface of the solution [13,14].
- **Phenols:** The presence of phenolic compounds was indicated by a dark blue coloration when the extracts were treated with ferric chloride [12,13].
- **Carbohydrates:** Molisch's test showed a positive result for carbohydrates in both leaf and flower extracts, indicated by the formation of a violet ring at the interface [14].

The presence of alkaloids, flavonoids, saponins, tannins, terpenoids, glycosides, phenols, and carbohydrates in *Bougainvillea spectabilis* extracts suggests that the plant may possess a wide range of pharmacological activities. Alkaloids are known for their analgesic, anti-inflammatory, and antimicrobial properties. Flavonoids exhibit antioxidant, anti-inflammatory, and anticancer activities. Saponins possess antidiabetic, immune-stimulating, and antimicrobial effects. Tannins have been reported to have anti-inflammatory, antiviral, and antioxidant properties. Terpenoids are known for their antimicrobial and anticancer properties. Glycosides may exhibit heart-protective effects, while phenols are renowned for their antioxidant activity. Carbohydrates contribute to the overall nutritional and energy values of plant extracts. These findings provide a scientific basis for the traditional use of *Bougainvillea spectabilis* in treating various ailments such as inflammation, infections, and gastrointestinal issues. However, further detailed studies including quantitative analysis, toxicity testing, and in vivo evaluations are necessary to fully explore the therapeutic potential of this plant [3.4.7.11].

Table 3 Percentage of Cardiac Tissues Working for Zebrafish (*Danio rerio*) [16]

		Zebrafish (*Danio rerio*)					
		Zebrafish 1	**Zebrafish 2**	**Zebrafish 3**	**Zebrafish 4**	**Zebrafish 5**	**Zebrafish 6**
Animal Groups	**Control**	93%	98%	100%	95%	99%	100%
	Hypertensive	20%	23%	29%	18%	25%	21%
	Positive control	61%	59%	68%	65%	59%	63%
	Test-1	62%	57%	69%	65%	61%	63%
	Test-2	74%	78%	71%	79%	82%	88%
	Test-3	97%	93%	99%	95%	97%	96%

3.4. Animal Study

The behavioural activities of all the groups were compared. It was observed that the hypertensive group exhibited lethargy, whereas the positive control and all test groups showed increased hyperactivity in swimming behaviour. Histological analysis revealed that the test groups demonstrated improved cardiac tissue health compared to the hypertensive group [15,16]. The percentage comparison of cardiac tissue health across all groups is as follows:

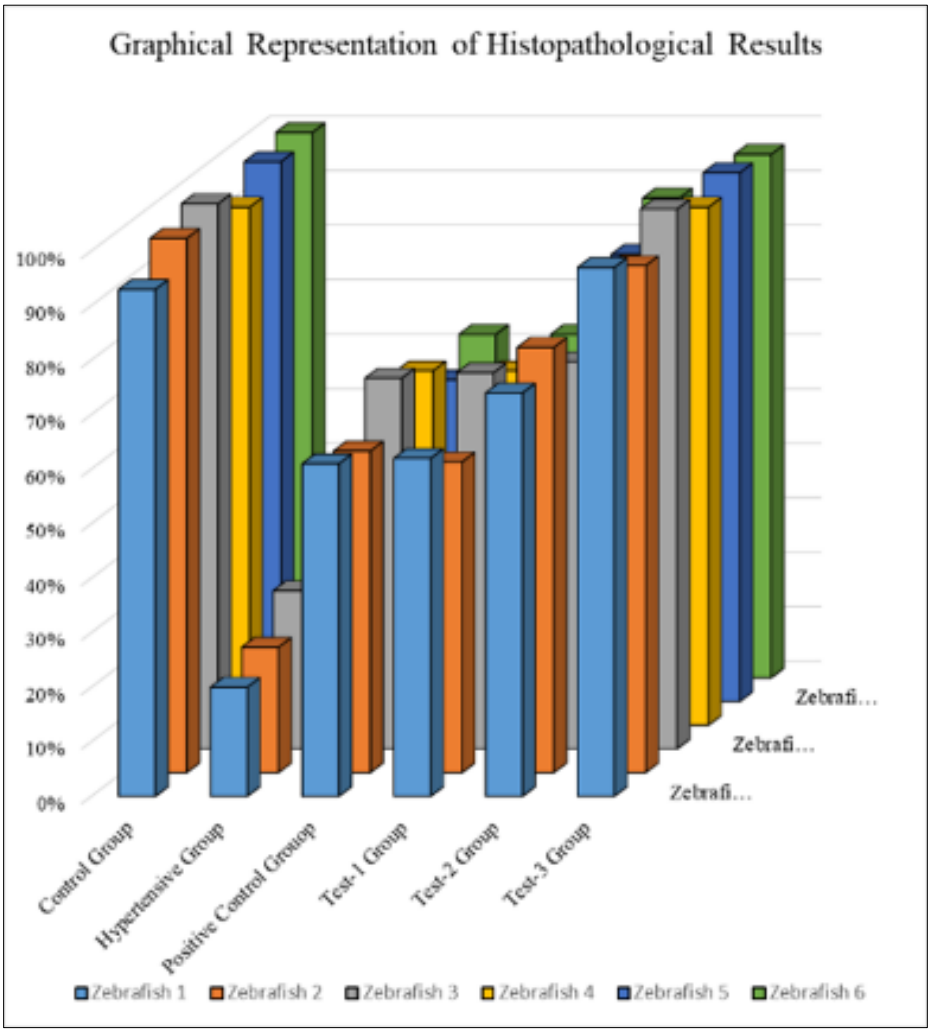


Figure 2 Graphical Comparison of Histopathological results of extract and drug treatments

3.5. Formulation and Development

The absorbance of the formulated nanoparticles was measured, and a peak was observed at 450 nm, confirming the formation of silver nanoparticles. The size of the nanoparticles ranged from 80 to 100 nm, and their shape was observed to be round [17].

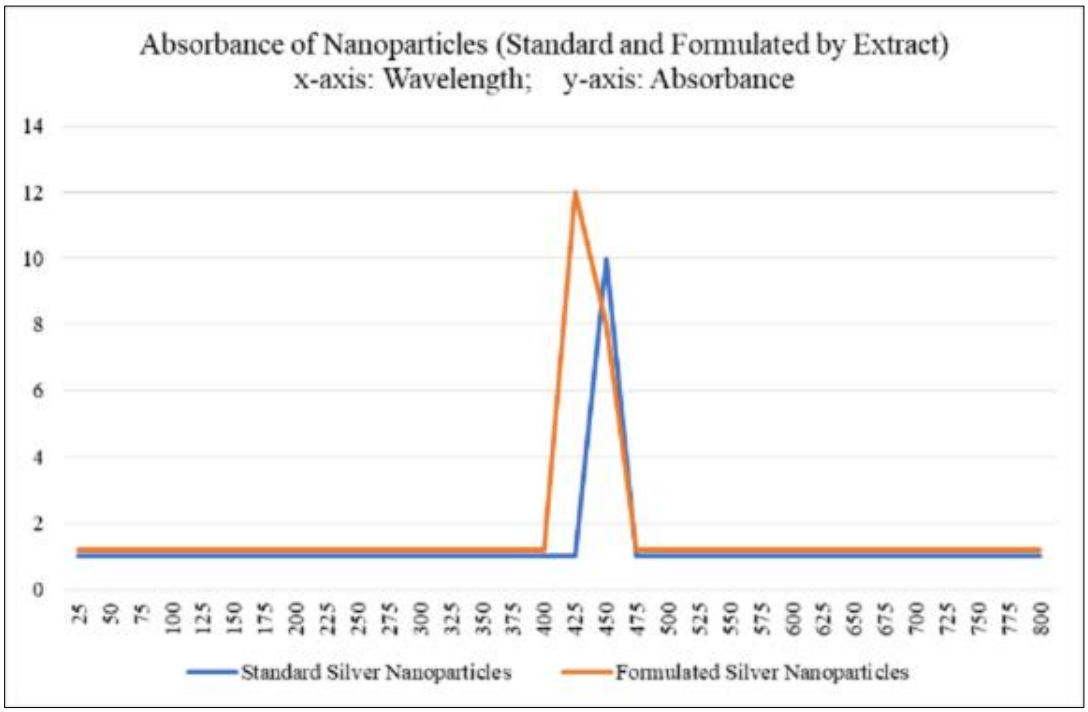


Figure 3 Comparative Analysis of Absorbance of Standard and Formulated Nanoparticles

4. Discussion

The yield obtained was more by Soxhlet as compared to maceration. Previous studies proved that the extract has various medicinal effects like anti-inflammatory, antimicrobial and antidiabetic activities. This experimental study proved that the plant extract can also be used for antihypertensive effect. The patients those who are using synthetic drugs they can only prevent hypertension, but this study has proved that the extract is treating the hypertension in the fishes. Though, the genomes of zebrafish and human have 90% similar genes so that this extract may also be effective in humans to treat hypertension [7,11,16,17].

5. Conclusion

Synthetic drugs can prevent the hypertension, but they cannot treat it. The extract of *Bougainvillea spectabilis* has proven that it can treat the hypertension by normalizing its pathophysiology.

Future directions

Different formulations should make for the extract of *Bougainvillea spectabilis* which will be easy to administer, and its clinical studies should perform for its specific pharmacological action and mechanism of action.

Compliance with ethical standards

Acknowledgments

The experimental work was performed at the Science Laboratory of Supreme Global School and the funds have been provided by the management of Supreme Global School, Aurangabad.

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The animal (zebrafish) used in the study was handled according to all the guidelines given by Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA).

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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