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PHYTOCHEMICAL CONSTITUENTS AND ANTICANCER ACTIVITIES OF CRUDE LEAF EXTRACT OF *BOUGAINVILLEA SPECTABILIS*

Short title: Applications of crude leaf extract of Bougainvillea spectabilis

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Abstract: Plants are a rich source of chemotherapeutics and can provide valuable bioactive molecules that contribute in the treatment of a variety of diseases, including cancer. *Bougainvillea spectabilis*, often known as big Bougainvillaea, is a flowering plant that has been used as an anti-inflammatory, antidiabetic, antimicrobial, and anticancer agent in traditional medicine. The goal of this work was to conduct a phytochemical analysis and test the anticancer efficacy of *B. spectabilis* crude leaf extract against the A549 lung cancer cell line. The phytochemical contents of the dried powdered plant leaf were extracted using the soxhlet and rotary evaporator with aqueous and organic solvents (acetone, chloroform, ethyl acetate, ethanol, methanol and water). Phytochemical analysis of *B. spectabilis* crude leaf extract revealed the presence of alkaloids, flavonoids, glycosides, phlobotannins, saponins, steroids, tannins, and terpenoids. Furthermore, the crude leaf extract inhibited cancer cells and had moderate anticancer efficacy against A549 cancer cell lines.

***Index Terms – Bougainvillea spectabilis*, Leaf crude extract, Phytochemicals, Anticancer applications**

I. INTRODUCTION

Cancer is a major public health issue that affects both developed and developing countries around the world [1]. In 2020, an estimated 19.3 million new cancer cases (18.1 million excluding nonmelanoma skin cancer) would be diagnosed globally, with around 10.0 million cancer deaths (9.9 million excluding nonmelanoma skin cancer) [2]. Given the disease's high notoriety, treatment has been a never-ending battle with mixed results. Surgical removal and radiation treatment of the vast accumulated biomass of cancer are now available alternatives for cancer treatment, which are often followed by systemic chemotherapy treatment for maintenance [3]. Antimetabolites, DNA-interactive agents (e.g., cisplatin etc.), anti-tubulin compounds (taxanes), hormones, and molecular targeting agents are the most commonly used chemotherapeutic drugs [4]. Chemotherapy's main drawbacks are cancer recurrence, drug resistance, and harmful effects on non-targeted tissues, which might limit the use of anticancer medications and hence lower the patient's quality of life [5]. The hunt for new promising anticancer medicines with improved efficacy and fewer side effects continues to overcome the difficulties of current therapy.

Plant-derived phytochemicals and derivatives have the potential to increase cancer patients' treatment efficacy while reducing side effects [6,7]. Several of these phytochemicals are physiologically active substances found in nature that have anticancer properties [8]. The testing of natural extracts for potential anticancer biological activity, followed by purification of active phytochemicals based on bioassay-guided fractionation and testing for in vitro and in vivo effects, is the first step in the development of effective and side-effect-free phytochemical-based anticancer therapy [9]. Given the enormous potential of *Bougainvillea spectabilis* as a source of medicines (Figure 1), researchers conducted a thorough search for phytochemicals in leaf extract and their anticancer effects, notably in the non-small-cell lung carcinoma (NSCLC) A549 cell line [10].

The leading causes of cancer-related death worldwide are small cell lung cancer and non-small cell lung cancer [11]. Non-small cell lung cancer is the most common type of lung cancer, accounting for 85–90% of all occurrences [12]. Tobacco smoking is the most common cause of non-small cell lung cancer. Furthermore, Kras and EGFR molecular activation, as well as p53 inactivation, are prevalent genetic changes that contribute to non-small cell lung cancer development [13,14]. For non-small cell lung cancer, first-line platinum-based chemo medicines have remained the standard treatment [12]. The use of metal-based anticancer compounds in lung and other cancer research has recently

gotten a lot of interest. Many studies have focused on countering the negative effects of platinum compounds since the development of platinum anticancer complexes [15,16]. For the treatment of cancer, various therapies have been proposed, many of which employ plant-derived compounds. The vinca alkaloids (vinblastine, vincristine, and vindesine), epipodophyllotoxins (etoposide and teniposide), taxanes (paclitaxel and docetaxel), and camptothecin derivatives are the four types of plant-derived anticancer drugs now on the market (camptotecin and irinotecan) [16]. Plants, as a reservoir of natural compounds with chemoprotective potential against cancer, nevertheless have a lot of promise to provide innovative treatments. As a result, the primary goal of this study was to look into the presence of alkaloids, flavonoids, glycosides, phlobotannins, saponins, steroids, tannins, and terpenoids in the crude leaf of *B. spectabilis*. In addition, the capacity of crude leaf extract to inhibit cancer cells, particularly A549 cancer cell lines, was tested.

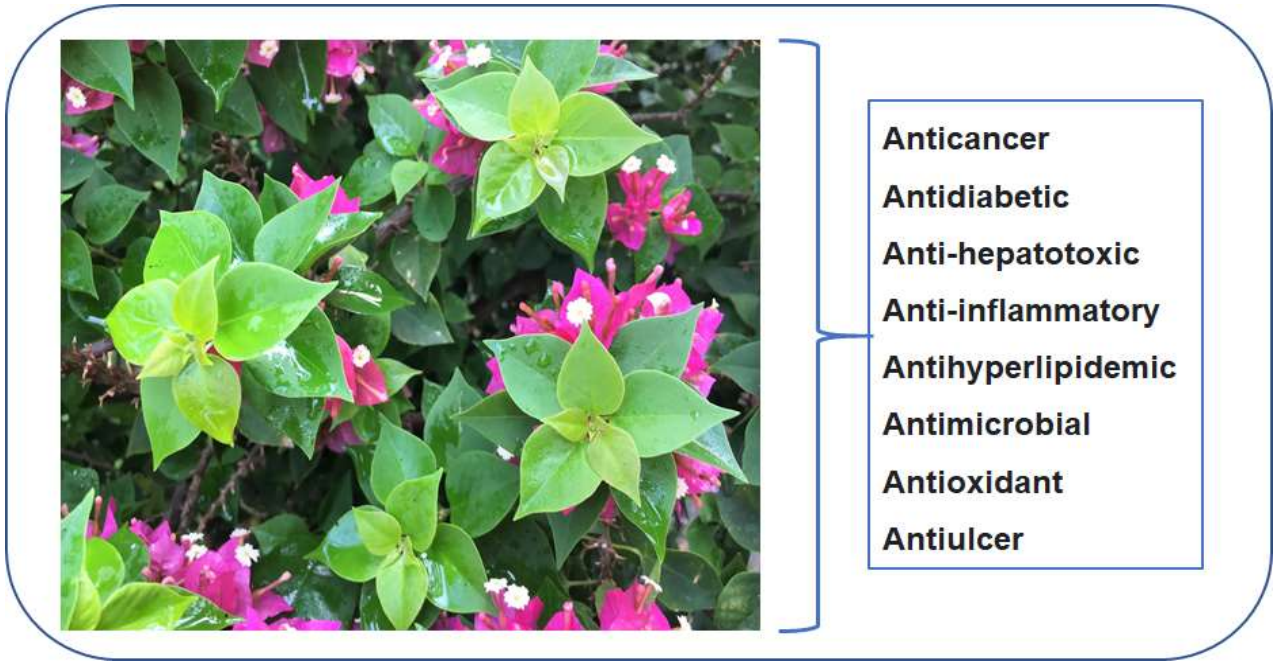

Figure 1. Morphology of the plant, *Bougainvillea spectabilis* and its pharmaceutical properties.

II. METHODOLOGY

Plant material and collection:

The leaves of *B. spectabilis* were collected from Tirumala-Tirupati forest areas of Chittoor District, Andhra Pradesh, India. Qualitative screening for pectinase producing bacteria:

Phytochemical Extraction:

Collected leaves were thoroughly washed with distilled water to remove the dust particles. The thoroughly washed leaves were air-dried at room temperature for a week, then ground into fine powder and stored in a dry air tight container to avoid further contamination. 5g of fine powder of leaves of *B. spectabilis* was transferred into a beaker and 100 ml of each acetone, chloroform, ethyl acetate, ethanol, methanol and water was separately added and allowed to stand for 48h and then filtered. The mixture was filtered using Whatman No. 1 filter paper. The filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55°C.

Phytochemical studies:

Preliminary phytochemical screening was carried out by using standard procedures described by Harborne [17].

Detection of alkaloids:

To detect the presence of alkaloids, whole leaf extract collected from various tested solvents of *B. spectabilis* were dissolved individually in dilute Hydrochloric acid (HCl) and filtered.

a) **Mayer's Test**: Filtrates were treated with Mayer's reagent ($HgCl_2$-1.36 gm. in 60 ml dist. Water; KI-5 gm. in 10 ml dist. Water; H_2O_2-30 ml). Formation of a yellow colored precipitate indicates the presence of alkaloids.

b) **Wagner's Test**: Filtrates were treated with Wagner's reagent (Iodine - 1.27 gm; KI - 2 gm; Distilled water - 100 ml). Formation of brown/reddish precipitate indicates the presence of alkaloids.

c) **Dragendroff's Test**: Filtrates were treated with Dragendroff's reagent, which was a mixture of potassium bismuth iodide prepared from basic bismuth (III) nitrate [(Bi $(NO_3)_3$ $5H_2O$ — 8 gms in 20 ml liquid NH3]; and potassium iodide (KI) [27.2 gm. in 50 ml dist. Water]. Formation of red precipitate indicates the presence of alkaloids.

***Detection of carbohydrates*:**

To detect the presence of carbohydrates, whole leaf extract of *B. spectabilis* isolated from different solvents was dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates.

a) **Molisch's Test**: In Molisch's test, when concentrated hydrochloric is introduced, the carbohydrate (if present) undergoes dehydration, resulting in an aldehyde formation. This aldehyde undergoes condensation along with two phenol-type molecules (such as ∝-naphthol, resorcinol, and thymol), resulting in a complex of purple or reddish-purple colours. Hence the filtrates of FWEE were treated with 2 drops of alcoholic α-naphthol solution in a test tube. The formation at the junction of the purple or reddish-purple ring indicates the presence of carbohydrates.

b) **Tests for Glycosides**: Most plants store chemicals in the form of inactive glycosides, which can be triggered by hydrolysis of the enzyme, which allows the sugar component to be broken off, making the chemical available for use. Many of those plant glycosides are used as medicines and can be detected by Liebermann's Test.

c) **Liebermann's Test**: The Liebermann test was studied for glycoside detection and for this we have added 2.0 ml of acetic acid and 2 ml of chloroform to the leaf extract of extracted from different solvents. The mixture was then cooled and added to concentrated sulfuric acid. Formation of green colored indicates the entity of aglycone, steroidal part of glycosides.

d) **Test for Tannins**: The whole leaf extract powder (0.30 g) of *B. spectabilis* was weighed in a test tube and boiled for 10 minutes in a water bath with 30 cm3 of water. Using the Whatman filter paper, filtration was performed after boiling. 3 drops of 0.1 per cent ferric chloride was added to 5 cm3 of the filtrate. Positive test showed a brownish green or a blue-black colouration.

e) **Test for Saponins**: 5.0 ml of distilled water was mixed with leaf extract powder (10 mg) of *B. spectabilis* in a test tube and it was mixed vigorously. The frothing was mixed with few drops of olive oil and mixed thoroughly and the foam appearance showed the presence of saponins.

***Test for Proteins*:**

a) **Biuret Test**: The Biuret test is a chemical test which can be used in a given analyte to search for the existence of peptide bonds. The presence of peptides in this study results in the formation of light purple colored copper (II) ion coordination compounds. Leaf extract solution was treated with 10% sodium hydroxide solution and two drops of 0.1% copper sulphate solution and observed for the formation of light purple color.

Test for Steroids:

a) Plant steroids are a diverse variety of natural products and they are derived via acetate-mevalonate pathway from S - squalene - 2,3 - epoxide. Phytosterols are omnipresent in the plant kingdom amongst plant steroids. 2 ml of chloroform and concentrated H_2SO_4 were added with the 5 ml aqueous leaf extract powder solution of *B. spectabilis*. In the lower chloroform layer red color appeared that indicated the presence of steroids.

Tests for Flavonoids:

Flavonoids are a group of plant metabolites that are thought to provide health benefits through signaling pathways and antioxidant activity in cells.

a) **Shinoda Test**: Pieces of magnesium ribbon and concentrated HCl were mixed with aqueous leaf extract powder solution of *B. spectabilis* and after few minutes formation of pink colour showed the presence of flavonoid.

b) **Alkaline Reagent Test**: 2 ml of 2.0 % NaOH mixture was mixed with *B. spectabilis* aqueous leaf extract powder solution; then concentrated yellow colour was produced, which became colourless when 2 drops of diluted acid were applied to the mixture. The positive outcome indicated the presence of flavonoids.

***Detection of phenols*:**

Phenolics are chemicals with one or more aromatic rings containing one or more hydroxyl groups. They are widely distributed in the plant kingdom and are the most common secondary plant metabolites, ranging from simple molecules such as phenolic acids to strongly polymerized substances such as tannins

a) ***Ferric Chloride Test***: In a tube containing 3 drops of *B. spectabilis* aqueous leaf extract powder solution, about 20 drops of 5 percent $FeCl_3$ solution were added and the tube was then stirred. Positive test for phenols is an intense colour which ranges from purple to reddish brown.

Test for triterpenoids:

Triterpenoids are carbon-skeleton compounds based on six isoprene units biosynthetically derived from the hydrocarbon C30 acyclic, Squalene.

a) **Liebermann Burchard test** - *B. spectabilis* aqueous leaf extract powder solution was mixed with few drops of acetic anhydride, boiled and cooled. The concentrated sulphuric acid was then added from the side of the test tube and observed at the junction of two layers for the formation of a browning. Formation of deep red colour in the lower layer would indicate a positive test for triterpenoids.

Cell culture:

A549 and Human dermal fibroblast cells (HDFa) cells were obtained from ATCC, cultured in DMEM, and supplemented with 10% FBS and 1% penicillin-streptomycin solution. The cells were grown in 37°C and 5% CO_2 and sub-cultured after reaching 90% confluency.

Anticancer properties of crude leaf extract of B. spectabilis by MTT assay:

The A549 and HDFa cell lines were seeded in 96-well tissue culture plates and incubated at 37^0 C with 5% CO_2. When the cells reached 50% confluency, A549 cells were treated with various concentrations of crude leaf extract ranging from 0, 20, 40, 60, 80 and 100 µL. Similarly, HDFa cells were also treated with various concentrations of crude leaf extract ranging from 0, 20, 40, 60, 80 and 100 µL and incubated at 37^0 C with 5 % CO_2 for 48 hours. Following treatment, cell viability was determined by MTT assay [18].

III. RESULTS AND DISCUSSION

Phytochemical screening of *B. spectabilis* leaf extract was performed to detect either the presence or absence of secondary metabolites such as alkaloids, phenolic compounds, flavonoids, glycosides, quinones, saponins, tannins, triterpenoids, etc. and the results were shown in **Table 1**. Phytochemical screening disclosed the existence of several significant bio compounds such as alkaloids, carbohydrates, flavonoids, steroids, glycosides, proteins, tansnins, saponins, and phenols known because of significant biological activity. Most of the derived compounds are compounds that have been known to be biologically active by various pathways and exert antimicrobial, analgesic, antipyretic, anti-inflammatory, antimicrobial and antioxidant activities. To our knowledge, this is the first study of its kind on the chemical composition of *B. spectabilis* leaves water extract, revealing an abundance of biologically relevant compounds such as alkaloids, tannins, flavonoids, quinones, terpenes, saponins, and so on. Isolating particular phytochemical elements and subjecting them to biological activity, on the other hand, would almost certainly yield favorable impact.

Table 1 Qualitative analysis of phytochemical constituents of the leaf extract of *B. spectabilis* extracted from various solvents: (A) water (B) acetone (C) chloroform (D) ethyl acetate (E) ethanol (F) methanol

S.No	Phytochemical constituents	Chemical test	A	B	C	D	E	F
1.	Alkaloids	Mayer's test	-	-	-	-	+	-
		Wagner's test	+	+	+	+	+	+
		Dragendroff's test	-	-	-	-	-	-
2.	Carbohydrates	Molisch’s test:	+	+	+	-	+	+
3.	Glycosides	Liebermann’s test.	+	+	+	+	+	+
4.	Tannins	Ejikeme test	-	-	-	-	-	-
5.	Saponins	Foam test	+	+	+	-	+	+
6.	Proteins	Biuret’s test:	+	+	+	-	+	+
7.	Flavonoids	Shinoda test	-	-	-	-	-	-
		Alkaline test	+	+	-	+	-	+
8.	Phenols	Ferric chloride test	+	+	+	-	+	-
9.	Steroids	Libermann - Burchard's test	+	-	+	-	+	+
10	Triterpenoids	Libermann - Burchard's test	-	+	-	-	+	-
		Salkowski's test	-	-	-	+	-	-

Figure 2 depicts the impact on cells after 24 hours of treatment with crude leaf extract at various concentrations. With increasing time of leaf crude extract treatment, the number of viable cells dropped and the number of dead cells grew. Cells treated with crude extract had a time-dependent decrease in viability. In the A549 cell line, treatment with crude extract reduced the number of live cells during the course of 24 hours of incubation. Even after 24 hours of treatment with the crude extract, HDFa cells demonstrated a greater survival rate, with reduced or no cell death in the healthy cell lines. Overall, increasing concentrations of leaf crude extract caused more toxicity and cell death in the A549 cell line, while higher concentrations caused minimal cell death in the HDFa cell line. Figure 2 shows the average values of three experiments. For the assessment of cytotoxic effects using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, A549 and HDFa cells were treated with crude leaf extract of water at different concentrations. The leaf crude extract have shown better cytotoxic effects and the obtained results from MTT assay has shown the IC50 value at 60 µL mL^{-1} concentration of crude extract in A549 cells where 50% of the cell proliferation was inhibited. It therefore indicates that leaf extract of *B. spectabilis* extracted from water has the potential for cytotoxicity to A549 cell lines and has significantly inhibited the proliferation of A549 cell lines by dose-dependent. However, healthy cell lines (HDFa) have less of an impact from *B. spectabilis* leaf extract extracted from water, confirming the extract's efficacy as a cancer-fighting drug with few side effects on healthy cells.

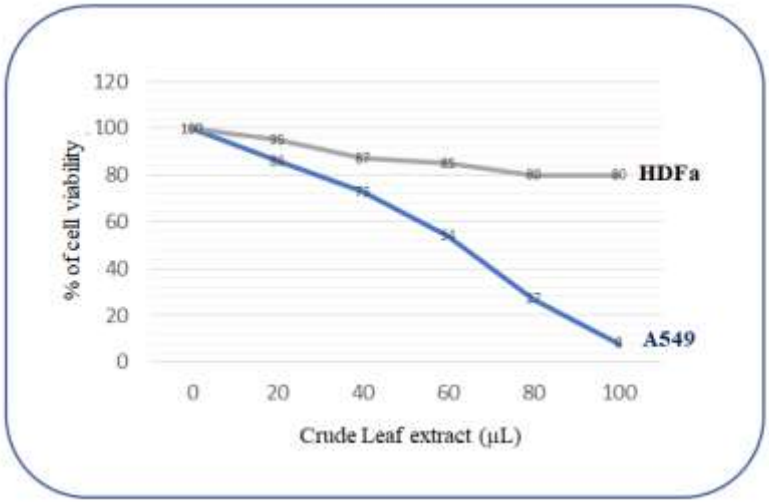

Figure 2. After 24 hours of treatment with crude leaf extract at various concentrations, the effect on cells (A549 and HDFa)

IV. SUMMARY AND CONCLUSION

In conclusion, the plants are a rich source of chemotherapeutics and can provide valuable bioactive molecules that contribute in the treatment of a variety of diseases, including cancer. As a result, the purpose of this study was to perform a phytochemical analysis and investigate the anticancer activity of *B. spectabilis* crude leaf extract against the A549 lung cancer cell line. The phytochemical components of the dried powdered plant leaf were extracted with aqueous and organic solvents using the soxhlet and rotary evaporator. Alkaloids, flavonoids, glycosides, phlobotannins, saponins, steroids, tannins, and terpenoids were discovered in *B. spectabilis* crude leaf extract of water aquaeous solution, water. Furthermore, against A549 cancer cell lines, the crude leaf extract suppressed cancer cells and demonstrated modest anticancer effectiveness without damaging healthy cells.

V. Acknowledgment

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