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Polyphenolics of *Plumeria rubra L.and* Cytotoxic Activities against Lung, Colon, and Breast Carcinoma

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Abstract

Numerous polyphenolic compounds originating from plants are thought to have anticancer and apoptosis-inducing effects on cancer cells. *Plumeria rubra* (family Apocyanaceae) is utilized as traditional medicine to cure a variety of diseases. It is well known for being a decorative tree, broad cultivated, very fragrant, and having a wide range of secondary metabolites, which inspired us to learn more about its possible medical uses. In this work, the total phenolic content of the methanol extract of *P. rubra* was established spectrophotometrically and found to be 11.05 mg/g, while the total flavonoid content was found to be 29.22 mg/g. Fifteen polyphenolic compounds were identified by the HPLC analysis of *P. rubra* methanol leaves extract. Additionally, an *in vitro* cell viability test was used to assess the cytotoxic activity of the methanol leaves extract of *P. rubra*. The findings of the cytotoxicity test revealed substantial anticancer activity against the human lung carcinoma A-549 cell line with an IC_{50} of 41.72 µg/ml and less activity against the human colon carcinoma HCT-116 and the human breast carcinoma MCF-7 cell line with IC_{50} of 57.62 and 75.8 µg/ml, respectively. Results of this work demonstrate that methanol leaves extract of *P. rubra* is a valuable source of phenolics and flavonoids that that can substantially slow the spread of cancer.

1. Introduction

Cancer is one of the top causes of mortality for people globally. Malignant cells are created because of gene mutations in one or more genes. Cancerous cells leave tumors and disperse to other sections of the body via the lymphatic or circulatory systems through a process known as metastasis [1]. Since any type of cell can develop cancer, there are numerous cancer types. Lung, breast, stomach, and colorectal cancer were the four most prevalent types of cancer described for two-fifths of the total instances of cancers detected worldwide. In low- and middle-income nations, cancer deaths accounted for more than 70% of all deaths [2]. Depending on the type and stage of the cancer, various therapeutic options are available for the therapy. Surgical, chemotherapy, and radiation therapy are the major components of the conventional treatment of cancer. Due to accompanying side effects, the potential emergence of multidrug resistance, and the high cost of care, the present cancer therapy has certain limits. The effects of several herbs on cancer patients have been the subject of numerous scientific investigations. While some treatments have been demonstrated to lessen the negative effects of cancer treatment [3].

Plumeria, a genus in Apocyanaceae family, are usually cultivated in gardens, for their showy and fragrant flowers [4]. Plumeria rubra is an ornamental plant, a small, deciduous spreading tree having a height of about 7-8 m. *P. rubra possesses a long history, and because it was once grown close to temples, people would often refer to it as a "temple tree."* Different classes of bioactive compounds have been reported in P. rubra L., including iridoids, terpenoids, flavonoids, glycosides, phenolics, alkaloids, fatty acids, esters, amino acids, steroids [5]. Although different biological activities have been reported on various parts of the plant, including asthma, constipation, abortifacient, diabetic mellitus, leprosy, inflammation, ulcers, wound healing, earache, pain, and many other things, a little research has been documented regarding anticancer activity of P. rubra. For that reason, we chose three cell lines viz; A-549, HCT-116, MCF-7 to be investigated by the methanol leaves extract of P. rubra. Lately, phenolic compounds have obtained significant interest based on their effect in holding back a variety of human illnesses. It is well-known that they are a category of compounds naturally found in plant foods, such as fruits, vegetables, and spices. They are also thought to reduce inflammation, which is thought to be the root cause of many chronic illnesses. They can neutralize harmful free radicals that would otherwise damage your cells and increase your risk of conditions like cancer, diabetes, and heart disease [6]. The aim of this study is to explore of P. rubra main phytoconstituents; via spectrophotometric determination of total phenolic and flavonoids also; investigation of the qualitative and quantitative polyphenolic compounds using RP-HPLC analysis; as well as to assess the in-vitro cytotoxic activities of the methanol leaves extract against human colon carcinoma HCT-116, human lung carcinoma A-549 and human breast carcinoma MCF-7 cell lines.

2. Experimental

2.1. Plant material:

The fresh leaves of *P. rubra* were collected during the flowering stage in June 2019 from the Experimental Station of Medicinal and Aromatic Plants, Faculty of Pharmacy, Cairo University, Giza, Egypt. The identity was identified by Professor Dr. Wafaa Mohamed Amer, Professor of Taxonomy, Faculty of Science, and Cairo University, Egypt. The plant was separately air-dried and kept in a tightly closed container. Voucher specimens of *P. rubra* was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, October 6 University (No. Pr. 2019-180).

2.2. Extraction:

Sample of the air-dried powdered leaves (0.5 kg) *P. rubra* was extracted by cold maceration in 70 % aqueous methanol till exhaustion and the solvent was evaporated under reduced pressure and low temperature (50°C) to yield dark green semi-solid sticky residue.

2.3 Preliminary Phytochemical Screening:

The extract was subjected to preliminary phytochemical tests followed by the methods of Harborne and Trease and Evans [7].

2.4 Determination of the total phenolic content (TPC):

The concentration of total phenolics of the methanol extract of *P. rubra* leaves was determined using the Folin–Ciocalteu method [8]. Gallic acid was administered as the standard for calibration curve preparation. Three replications were performed, and the results were expressed as mg of GAE (gallic acid equivalent) per 1g of the tested extract.

2.5 Determination of the total flavonoids content (TFC):

The concentration of the total flavonoid content of the methanol extract of *P. rubra* leaves was determined using the aluminum chloride colorimetric assay with slight modifications to be carried out in microplates [9].

2.6 RP HPLC analysis:

RP HPLC analysis was carried out using an Agilent 1260 series. The separation was carried out using (Eclipse C18) reversed phase column (4.6 mm × 250 mm i.d., 5 mm). The mobile phase consisted of water (A) and 0.05% tri□uoroacetic acid in acetonitrile (B) at a □ow rate of 1 mL min−1. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5–8 min (60% A); 8–12 min (60% A); 12–15 min (85% B) and 15–16 min (82% B). The multiwavelength detector was adjusted at 280 nm. The injection volume was 10 mL for each of the sample solutions. The column temperature was maintained at 35 °C [10].

2.7. Cytotoxic activity:

The American Type Culture Collection (ATCC, Rockville, MD) provided the cell lines for human breast carcinoma (MCF-7), lung carcinoma (A-549) and colon carcinoma (HCT-116). The cells were raised on RPMI-1640 media supplied with 10% foetal calf serum deactivated and 50 g/ml gentamycin. The cells were sub-cultured 2 to 3 times per week and kept at 37°C in a humid environment using 5% CO2. The MTT assay was used to estimate the quantities of live cells for anticancer experiments [11].

3. Results and Discussion

3.1. Phytochemical analysis

The preliminary phytochemical investigation of powdered leaves of *P. rubra* revealed the presence of alkaloids, phenolic compounds, flavonoids, steroids, terpenoids, coumarins, iridoids, carbohydrates, essential oils, and traces of tannins while saponins and anthraquinones were not detected.

3.2. TPC

The total phenolic content of the methanol leaves extract of *P. rubra* was evaluated as GAE concerning a standard curve. The phenolic content was determined spectrophotometrically at 630 nm which was found to be 11.05 mg of GAE/g (Figure 1).

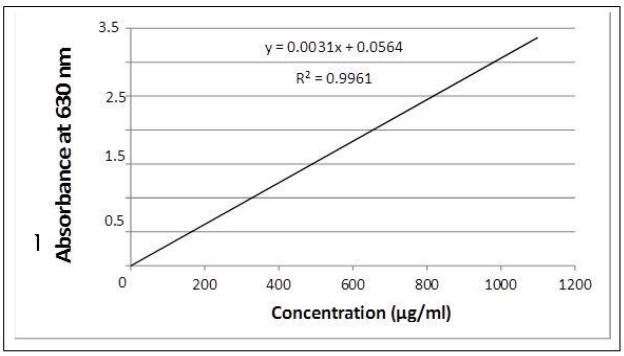


Figure (1). Calibration curve of gallic acid.

3.3. TFC

The total flavonoid content of methanol extract of *P. rubra* was determined spectrophotometrically at 420 nm, which was found to be 29.22 mg of RE/g using rutin calibration curve (Figure 2).

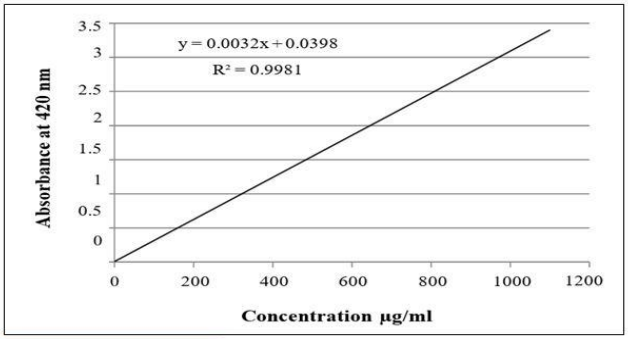


Figure (2). Calibration curve of rutin

3.4. RP HPLC analysis:

HPLC analysis of methanol leaves extract of *P. rubra* was studied using Reversed Phase-High Performance Liquid Chromatography. Concerning the polyphenolic compounds investigated in the leaves of *P. rubra*, coumaric acid was the major phenolic acid (95.6 µg/g) followed by syringic acid (35.2 µg/g). While rutin was the highest concentration as the flavonoid compound present (577.9 µg/g) followed by

naringenin (178.5 µg/g), results are compiled in Tables (1& 2) and (Figure 3).

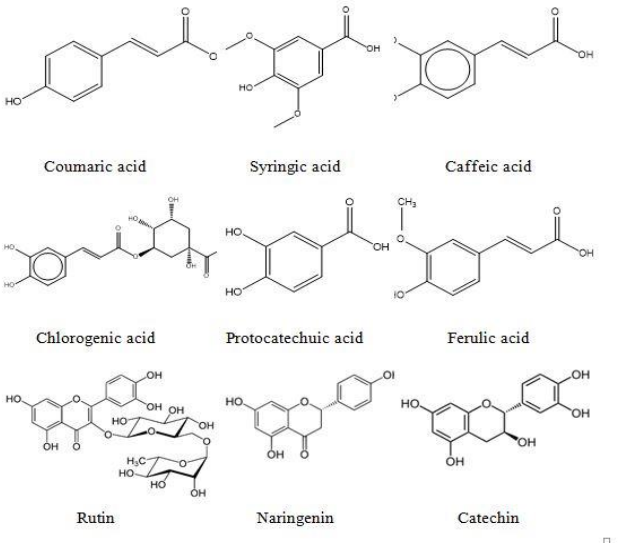


Figure (3). Chemical structures of the most abundant phenolic compounds in the P. rubra methanol extract

Table(1): RP-HPLC analysis of the phenolics in the MeOH extract of *P. rubra*

Compounds	Retention time (min.)	Peak Area (mAU×min)	Concentration of compounds (µg/g)
Chlorogenic acid	3.90	298.13	24.9
Gallic acid	3.09	40.65	3.8
Caffeic acid	5.43	584.28	22.4
Coumaric acid	**8.24**	**5283.78**	**95.6**
Ellagic acid	7.88	ND	ND
Protocatechuic acid	11.59	296.20	20.5
Vanillin	8.86	181.27	4.3
Cinnamic acid	13.27	662.01	6.7
Methyl gallate	5.03	348.11	4.5
Ferulic acid	9.50	344.89	12.1
Syringic acid	**5.93**	**797.85**	**35.2**

*ND: not detected

Table (2): RP-HPLC analysis of the flavonoids in the MeOH extract of *P. Rubra*

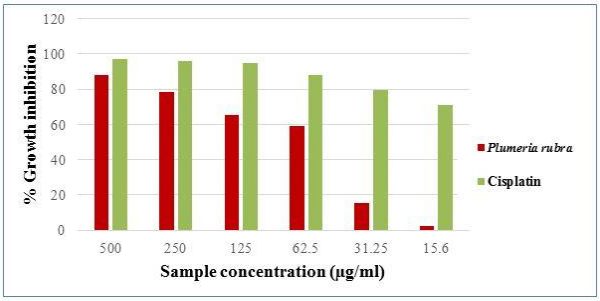
Compounds	Retention time (min.)	Peak Area (mAU×min)	Concentration of compounds (µg/g)
Rutin	**7.13**	**907.32**	**577.9**
Quercetin	9.40	ND	ND
Naringenin	**7.79**	**299.27**	**178.5**
Hesperetin	8.11	ND	ND
Apigenin	2.36	85.07	7.1
Diadzein	3.46	ND	ND
Catechin	4.33	185.8	40.30
Luteolin	6.92	107.99	10.2
Myricetin	5.40	ND	ND
Kaempferol	14.17	ND	ND
Taxifolin	12.14	ND	ND

*ND: not detected

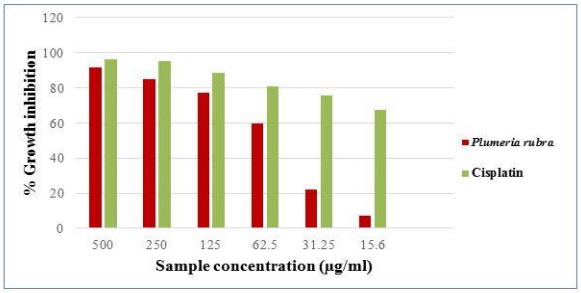
3.5 Cytotoxic Evaluation

The cytotoxicity of the different concentration of the *P. rubra* extract was estimated in terms of percent of growth inhibition compared to untreated control cells and their IC_{50} in µg/ml was detected; cisplatin resulted in a significant and dose-dependent decrease in the surviving fraction of human colon carcinoma HCT-116, human lung carcinoma A-549 and human breast carcinoma MCF-7 cell lines in the tested extract of *P. rubra* with IC_{50} 57.62, 41.72 and 75.8 µg/ml , respectively.

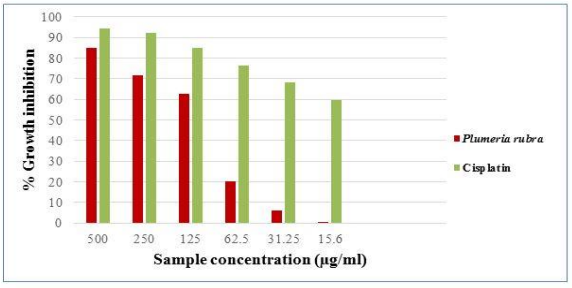
Anticancer screening results demonstrated in Figures (4-6) showed that the total methanol extract of *P. rubra* exerted a promising anticancer activity against lung carcinoma A-549 cell line to the standard drug cisplatin, with IC_{50} value (41.72 µg/ml). On the other hand, the extract of *P. rubra* exhibits a moderate anticancer activity against colon carcinoma HCT-116 cell line IC_{50} value was (57.62 µg/ml). As for breast carcinoma MCF-7 cell line to the reference drug cisplatin, showing IC_{50} value (75.8 µg/ ml).



Figure(4). Growth inhibition percent of the methanol leaves extract of *P. rubra* against colon carcinoma HCT-116 cell line compared to reference drug.



Figure(5). Growth inhibition percent of the methanol leaves extract of *P. rubra* against lung carcinoma A-549 cell line compared to reference drug.



Figure(6). Growth inhibition percent of the methanol leaves extract of *P. rubra* against breast carcinoma MCF-7 cell line compared to reference drug.

4. Discussion:

Cancer is the most common illness in developed countries and is the second-highest cause of mortality worldwide. Different malignancies affect various organs, including the brain, liver, lungs, kidney, breast, bones, and ovaries [12]. Even while medical technology has advanced significantly, there is still no global cure for almost all cancers. Medicinal plants provide the raw materials for natural remedies that are used to treat a variety of illnesses. There are many secondary plant

metabolites that offer medicinal and physiological effects, including flavonoids, alkaloids, saponins, phenolics, and terpenoids [13]. The anticancer effects of plant extracts and chemically made substances have been studied *in vitro*. Compared to the use of single, pure chemicals, natural product treatment has superior advantages. This may be because there are combinations of many curative or preventive ingredients present, which may be more effective in curing ailments than individual items are [14]. In this work, the phytochemical screening of a methanol leaves extract of *P. rubra* revealed the existence of various phytochemical classes, primarily phenolics, and flavonoids. According to the results of the phytochemical screening, the TPC and TFC of the methanol extract of *P. rubra* leaves showed that there were significant levels of phenolics and flavonoids present. Additionally, fifteen polyphenolic compounds were identified as ten phenolic acids and five flavonoids by comparison with the retention times of authentic standards investigated under identical conditions. A study found that plant phenolics are one of the major chemical classes that act as antioxidants and anticancer agents [15]. Therefore, it is important to investigate *P. rubra's* phenolic profile and antiproliferative effects. The cytotoxicity of different concentrations of the methanol extract of *P. rubra* leaves was assessed on three human cancer cell lines HCT-116, A-549 and MCF-7 with an IC_{50} of 57.62, 41.72 µg/ml and 75.8 µg/ml, respectively.

The extract is recognised as being effective against cancer cell lines when the IC_{50} value is less than 100 µg/ml [16]. Our findings from polyphenolic HPLC offered proof that provided evidence in support of the potential anticancer since the predominant phenolic acids were coumaric and syringic acids, and the highest flavonoids were rutin and naringenin. *p*-coumaric (*p*-CA) acids being the major compound; the phenolic hydroxyl group provides the hydrogen atoms for scavenging the ROS [17]. Numerous reports on use of *p*-CA for therapeutic benefits against cancer cell lines include antiproliferative effect; attenuates the process of amyloid in a concentration dependent manner [18]. Additionally, syringic acid may have anti-cancer properties via influencing specific molecules related to the cell cycle, functioning as an antioxidant, anti-mitogenic, and changing immune system activity. Cytotoxic effect of syringic acid has been explored in lung, breast, liver, and colorectal cancer cell lines with promising results [19]. The anticancer effect of rutin has been extensively investigated. Human leukaemia HL-60 cells, neuroblastoma LAN-5 cells, B16F10 lung cancer cells, and colorectal cell lines all exhibit different pathways of rutin-induced suppression of cancer cell proliferation [20, 21]. Naringenin also triggers apoptosis and arrests cell cycle in several tumour cell lines, involving breast tumour cells, mammary tumour cells, and prostate cancer cells [22]. As a result, the primary phenolic and flavonoid components in *P. rubra* boost their cytotoxic properties.

5. Conclusion

Our study revealed that *P. rubra* leaves extract has significant anticancer activity against the lung carcinoma A-549 cell line with IC_{50} 41.72 compared to the used reference drug cisplatin while; exhibiting moderate activity against the human colon carcinoma HCT-116 and breast carcinoma MCF-7 cell lines. HPLC analysis showed the presence of 15 polyphenolic compounds in the *P. rubra* extract to which; the anticancer activities may be attributed. Our finding should serve as an implement for the future research on the mechanisms underlying the plant cytotoxicity as well as the investigation of other pharmacological actions.

List of abbreviations
P. rubra: *Plumeria rubra*
TPC: Total phenolic content

TFC: Total flavonoids content
BHA: butylated hydroxyanisole

RP HPLC: Reversed Phase-High Performance Liquid Chromatography

Consent for publication

Not applicable

Declaration of competing interest

The authors declare no conflict of interest.

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Authors' contributions

H.A.E. and N.A.S., conceptualization, methodology, resources, writing original manuscript and reviewing. S.A.E collection, drying and extraction of the plant. E.M.E. supervision, review. All authors have read and permitted the final submitted manuscript.

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