INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 25, No. 6, p. 18-24, 2024
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Bioassay assessment of *Catharanthus roseus* flower flavonoids: *In vitro* study

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Article published on December 08, 2024

Key words: Cytotoxicity, Antibacterial activity, Free radical scavenging, *Catharanthus roseus*

Abstract

Catharanthus roseus commonly known as periwinkle has a rich story in traditional medicine. Researchers isolated two flavonoid compounds, Quercetin and Rutin from the methanolic extract of its flower. These compounds were evaluated for their cytotoxic, antibacterial and antioxidant properties. In the brine shrimp lethality bioassay, Quercetin exhibited quite potent activity with an LC_{50} (6.52) suppressing Rutin (5.05) µg/ml. Additionally, Quercetin demonstrated strong antibacterial effects against pathogenic microorganism compared to streptomycin, while Rutin displayed significant antioxidant activity with an IC_{50} 10.86µg/ml. In addition, this bioassay assessment of *Catharanthus roseus* flower flavonoids is firstly reported in the present study.

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Introduction

Medicinal plant products are essential for improving general health and reducing the negative effects of many chemotherapy drugs. There is good reason to be conducted about the possible medical benefits of plants (Kaushik *et al*., 2002). Board spectrum bioactivity in crude plant extract can be detected using general bioassays such as the free radical scavenging activity test (FRST), antibacterial screening (ANBS), and brine shrimp lethality bioassay (BSLT). These methods are inexpensive, simple to learn, and call for lillte in the way of test material. Both cytotoxicity and pesticidal actions are predicted by BSLT (Ghisalberti *et al*., 1993). First used in 1982, it has acted as a guide for a fractionation of potent cytotoxic and anticancer drugs (Meyer *et al*., 1982), including cis-annonacin from *Annona muricata* and trilobacin (Zhao *et al*.,1992) from the bark of *Animina triloba* (Rieser *et al*., 1996). The initial application of FRST was in 1958 for the measurement of antioxidant activity (Blois, 1958) and the identification of compounds that are potent in free radical scavengers, including vitamin C, vitamin E, flavonoids, carotenes, phenolic acids, phytic acids, and phytoestrogens (Jaleel *et al*., 2009). These substances lower the chance of contracting an illness (Bendich *et al*., 2001). According to clinical research, antioxidants found in tea, red wine, fruits and vegetables can greatly reduce the prevalence of chronic illness like heart disease and several types of cancers (Miller *et al*., 2000).

Catharanthus roseus popularly called as Madagascar periwinkle or Periwinkle, is a member of Apocyanaceae family of plants. This evergreen subshrub or harbeceous plant grows up to 1 meter tall (Jaleel *et al*., 2009). It has a rich story in traditional medicine, where it is administrated as cooling remedy (Huxley, 1992). Periwinkle is utilized for various purposes, including treating diabetes, fever, malaria, throat infections, and chest complaints. Additionally, it helps regulate menstrual cycles and acts as euphoriant (Ambusta, 1992).

All sections of plants are rich in indole alkaloids, making them a visible source. Interestingly the leaves contain essential antineoplastic alkaloids called Vincristine and Vinblastine, which are utilized in the treatment of lymphoma and leukemia. On the other hand, antihypertensive alkaloids such as reserpine, serpentine and ajmalicine are present in the roots (Mishra *et al*., 2001). These alkaloids are essential for managing both malignant and nonmalignant diseases, including illnesses of the platelets (Fransworth *et al*., 1968; Svoboda *et al*., 1975).

Previous phytochemical investigations have led to the isolation of several compounds from *Catharanthus roseus*, including Kaemferol, Quercetin, Syringetin glycosides, Malvidin, Petunidin, Hirsutidin derivatives. These pharmaceutically important alkaloids contribute to the plant's medicinal properties, with Vincristine and Vinblastine being key players in cancer therapy, while ajmalicin and serpentine serve as antihypertensive agents (Forsyth *et al*., 1957; Nishibe *et al*., 1995; Bruneton, 1999; Filippini *et al*., 2003). The *Catharanthus* plant yields a large number of alkaloids with medicinal significance. The monoindole alkaloids serpentine and ajmalicine are antihypertensive medications, and they are antineoplastic (Zhao *et al*., 2007; Rahman *et al*., 1983; Rahman *et al*., 1984; Rahman *et al*., 1984; Rahman *et al*., 1985; Rahman *et al*., 1988; Auriola *et al*., 1990).

The purpose of the work is to evaluate the cytotoxic, antibacterial and antioxidant characteristics of two flavonoid compounds that were isolated from the methanolic extract of *Catharanthus roseus* flowers: quercetin and rutin. We hope to verify the historical uses of this important plant for medical purposes by doing this.

Materials and methods

Collection of plant material

A voucher specimen (No. = 39512) is kept in storage at the Bangladesh National Herbarium in Dhaka, and the taxonomist there, recognized fresh blooms of *C. roseus* that were taken from the Botany Department gardens of Dhaka university in June 2021.

Experimental

The UV absorbance was measured using the Perkin Elmer Shelton, CT 06484 USA, Almbda 25 UV/VIS spectrometer. Liquids were evaporated using a vacuam rotary evaporator (BUCHI, Rotavapor R-210 Switzerland). In this study, all solvents used from Sigma-Aldrich, St. Louis, MO, USA (commercial sources).

Cold extraction (Preparation of the solvent extract)

Freshly *Catharanthus roseus* flowers were finely ground into powder using a grinding machine after being carefully dried at 38˚C in an oven. Powder of the flower (200g) was extracted successively different solvent at room temperature. At first it was extracted with n-hexane for 5 days and the extract was dried to get a gummy mass (7.15g) using Rotary evaporator. Then the residual part of the flower was extracted with dichloromethane for 5 days and the extract was dried to gummy mass (5.80g). Again the residual part was extracted with methanol and the filtrate was dried under reduced pressure to gummy mass (22.69 g).

Isolation of compounds from crude methanolic extracts

After being dissolved in a little amount of solvent, the dry mass of a 15.0 g methanol extract was adsorbed onto a silica gel of column grade. The substrate that has been adsorbed was subsequently layered over a TLC-grade silica inside a vacuum liquid chromatography (VLC) device. Following elution with 100% n-hexane and ethyl acetate (EA) mixtures, and methanol and ethyl acetate mixtures in increasing polarity, the system was then eluted. 26 conical flasks were used to collect in 200 ml portions. On the basis of their TLC (Thin Layer Chromatography) patterns, those collections were split into six portions.

Fraction 2 (collections 4-9), 3 (collections 10-13), 4 (collections 14-16), 5 (collections 17-19) and 6 (collections 20-25) were selected for further investigation to isolate pure compounds.

Compound (1), weighing 6.4 mg was isolated from the VLC fraction 4 (collection no mentioned above) using a medium sized column eluted with an ethyl acetate-methanol solvent systems gradient. The compound appeared as a yellow crystalline solid and was soluble in chloroform with a few drops of methanol. Compound (2), 7.60 mg was purified from the VLC fraction 5 (collection no mentioned above) by passing it through a column eluted with various ratios of ethyl acetate EA and methanol (MeOH) to increase polarity. The compound separated into yellowish crystals from collections 8 and 9 after standing overnight. The yellow crystalline compound 2, 7.6 mg was finally purified by washing with a few drops of n-hexane and EA. It exhibited solubility in methanol.

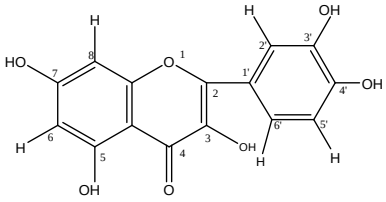

Fig. 1. 3,3',4',5,7-Pentahydroxyflavone or Quercetin (1)

Based on all spectroscopic data (Bauer *et al.*, 1996), literature values, and the melting point of the Compound (1), it was confirmed that the compound is 3,3′,4′,5,7-Pentahydroxyflavone, commonly known as Quercetin. The following is the compound (1)'s structure (Fig. 1).

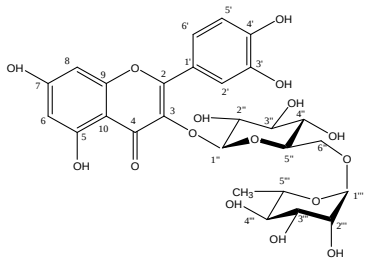

Fig. 2. Quercetin-3-O-rutinose or Rutin (2)

Based on all spectroscopic data (Oladimeji *et al.*, 2006), literature values and melting point of the compound, it was confirmed that the compound (2) is flavonoid glucoside named rutin. The following is the compound (2)'s structure (Fig. 2).

Bioassays of the isolated compounds

Antioxidant activity

The DPPH method was utilized to measure the antioxidant activity spectro-photometrically (Barry,

1980). The rich violet color of the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical is caused by its unpaired electron, and spectrophotometrically tracking of its scavenging activity can be achieved by the absorbance decreases at 517 nm. Several doses (from 5 µg/ml to 400 µg/ml in methanol) of an ascorbic acid solution (1ml) and flavonoid components extracted from methanol extract of *C. sophera* flowers (1ml) were combined separately with 3 ml of 0.4 mM DPPH solution in this investigation. Using a UV-Visible Spectrophotometer (Cintra, Australia), the solutions were dark-stored for thirty minutes before their absorbance at 517nm was recorded. Positive control was provided by ascorbic acid. Every test solution underwent a triplicate repetition of the entire process. Higher free radical scavenging activity was demonstrated by lower absorbance values in the reaction mixture. The degree to which the DPPH decolorization changed from purple to yellow indicated the extract's scavenging activity. The following formula was used to calculate the scavenging activity against DPPH: [(A-B)/A]×100, where, the absorbance of control DPPH solution without the sample is represented by the letter (A). Where, A is the absorbance of control (DPPH solution without sample). (B) Shows how much the DPPH solution absorbs when the sample-ascorbic acid or extract – is present. After plotting the scavenging activity (%) versus concentration, linear regression analysis was used to determine IC_{50} (Concentration 50% inhibition) value. Every procedure was run three times, and the outcome was averaged.

Cytotoxicity

The brine shrimp lethality bioassay method was utilized to evaluate cytotoxic activity. The test samples of the separated compounds were serially diluted at 150, 75, 37.5, 18.75, 9.375, 4.684, 2.344, 1.172, 0.586 and 0.292 µg/mL before being dissolved in dimethyl sulfoxide (DMSO). After adding one of these test solutions to a test tube with 10 shrimp in five milliliters of simulated brine water, the tube was allowed to sit at room temperature for a whole day. Following the incubation period, the Finney Method (Bauer *et al.*, 1996) was used to plot of proportion of affected shrimps versus the logarithm of the sample concentrations in order to calculate the test sample's median lethal concentration (LC_{50}). Vincristine sulphate, LC_{50}=0.57 was employed in this assay as a positive control to compare the test sample's cytotoxicity.

Antibacterial screening

Depending on how soluble they were, the test samples from isolated compounds were each independently dissolved in different amounts methanol or choloform. The disk diffusion approach was then used for antibacterial screening (Pietta, 2000; Rechner *et al.*, 2002). Using Streptomycin (10 µg/disc, Oxford, UK) as a standard, the diluted samples were applied to sterile discs (Oxford, UK) at a concentration of 100 µg/disc for this test.

Results and discussion

Fig. 3 represents the IC_{50} value of Quercetin and Rutin from flowers of *Catharanthus roseus*. The results showed the significant antioxidant activity of flavonoid compounds with IC_{50} value of 9.60 and 10.86 µg/ml, respectively which is very much comparable to the IC_{50} value (4.64 µg/ml) of positive control, L-ascorbic acid.

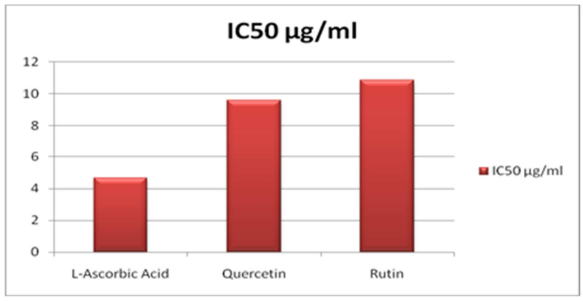

Fig. 3. MeOH extract of flowers *Catharanthus roseus* flavonoids in vitro antioxidant activity

According to the antioxidant function, Flavonoids quickly donate hydrogen atom to scavengers free radicals. Several investigations have examined the antioxidant potential of flavonoids with the goal of establishing a link between their molecular makeup and capacity to scavenge radicals. The configuration of their hydroxyl groups and molecular structure

determine how well flavonoids scavenge free radicals (Halliwell *et al.*, 1992). The availability of phenolic hydrogens and the possibility of stabilizing the ensuing phenoxy radicals via electron delocalization or hydrogen bonding are particularly important.

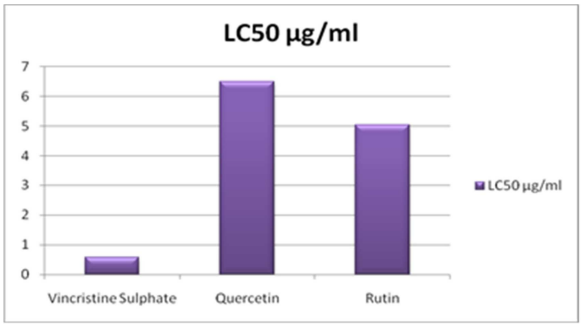

Fig. 4. MeOH extract of flowers *Catharanthus roseus* flavonoids cytotoxic effect on brine shrimp nauplii

Naturally occurring byproducts of metabolism are free radicals, and antioxidants are vital because they can stop or slow down the oxidation of substrates in chain reactions. Although synthetic antioxidants are commonly utilized, their limited use because of their harmful and carcinogenic effects has raised interest in natural antioxidants that don't have the same side effects (Jaleel *et al.*, 2007).

All flavonoids were found to have the ability to donate protons, even if their DPPH radical scavenging capacities were not as high as that of ascorbic acid. As main antioxidants, they might operate as scavengers or inhibitors of free radicals. Consequently, the chemicals that have been identified from *Catharanthus roseus* flowers show promise in treating free radical damage. Interestingly, the chemicals that were separated from flower methanol extracts showed stronger DPPH free radical scavenging activity (Saumya *et al.*, 2021).

The isolated compound's LC_{50} values for cytotoxicity, as determined by, brine shrimp lethality assay, were 5.05 µg/ml for rutin and 6.52 µg/ml for quercetin (Fig. 4). It is clear that all test substances were lethal to brine shrimp nauplii when compared to positive control, vincristine sulphate, which has an LC_{50} of 0.571 µg/ml. Remarkably, in the brine shrimp lethality experiment, both flavonoids from the flowers of *Catharanthus roseus* demonstrated noteworthy activity. These promising findings imply the possibility of their anticancer or pesticidal activity.

Table 1. MeOH extract of flowers *Catharnthus roseus* flavonoids (Antibacterial screening)

Test microorganism	Diameter of zone of inhibition (mm)		
	Quercetin	Rutin	Steptromycin
Gram-positive bacteria			
Bacellius cereus	6	6	16
Staphylococus aureus	7	8	15
Bacillus megaterium	11	10	21
Staphylococcus sp.	11	9	17
Gram-negative bacteria			
Vibreo colera	8	5	14
Escherichia coli	4	4	10

Using streptomycin (10 µg/disc) as a typical antibiotic disk, the antibacterial properties of quercetin and rutin from *Catharanthus roseus* flowers were screened at 100 µg/disc against a few harmful microorganism. Table 1 displays the outcomes.

Quercetin and Rutin, the two compounds under investigation, demonstrated a moderate zone of inhibition against almost all pathogenic bacteria. Furthermore, compared to gram negative bacterial species, theses active chemicals showed more effectiveness against gram positive bacteria. The different chemical makeup and cell wall structure of the two kinds of bacteria may be the cause of this activity difference.

Conclusion

The methanolic extract of *Catharnthus roseus* flowers was used to extract quercetin and rutin, which have antibacterial, cytotoxic, and antioxidant

characteristics. In addition, this bioassay assessment is firstly reported in the present study. These features are consistent with the traditional uses of this plant by the local communities. To investigate other isolated chemicals from this medicinally significant plant and evaluate their active phyto components, more research is necessary.

Acknowledgements

The researchers of this study would like to express special thanks to department of Pharmacy, Dhaka University for conducting bioassays of the crude extract of plant materials.

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