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Investigation of Bio-Physical and Chemical Characteristics of Wild Rose (*Rosa brununii* Lindl.) in Murree District, Pakistan

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Abstract

The present study was conducted to evaluate the vitamin C estimation, phytochemical screenings, antioxidant activity, and antimicrobial assay of the wild rose (*Rosa brununi*i Lindl.). Rose hips of this wild plant were collected from five different locations in Murree hills (Bhurbhan, Lower Topa, Ghoragali, Kotli Sattiyan, and Danoi). The concentration of Vitamin C was found in the range of 2000-2500 mg/100g. The phytochemical screening showed the presence of total flavonoid compounds in the range of 2.44-5.76mg/100g and the total phenolic contents were found in the range of 0.18 1.60mg/100g. Results showed that the subject plant exhibited no significant antifungal activity. *Rosa brununii* were screened for insecticidal activity against *T. castaneum*, *R. dominica,* and *C. analis*. And no activity against any of the test insects indicating that this genus has no insecticidal potential. *Lemna minor* L plant was used to detect the phytotoxic activity and results showed that at the concentration of 1000 and 100 µg/ml, the oils showed moderate activity of 50 and 40% respectively. At the concentration of 10 µg/ml, the sample showed low activity of 25%. Brine shrimp (*Artemia salina*) lethality bioassay was used to check the cytotoxic effect of *R. brununii* Lindl. and results revealed very low toxic effects of cytotoxicity.

Keywords: Cytotoxicity, phytochemical peculiarities, Wild rose

Introduction

Members of the wild rose family are high in vitamin C content and well-thought-out as one of the richest plant sources available. The seed of Rosehips plants comprises oil mainly unsaturated fatty acids 2.7 7.1% (Kazaz *et al.,* 2009), 6.9-8.6% protein and the quantity of Vitamin C is 0.22-0.44 mg per 100 g of rosehips seeds (Cinar & Dayısoylu 2004). Due to its elevated quantity of linoleic and linolenic acids, the valuable seed oil is used in cosmetics used for the cure skin and hair problems. Oil of Rosehip is highly useful in sun shield items such as sunblock. Rosehips having natural retinoic acid are useful remedies for eye diseases and skin-related problems, also used in pesticides. The recent studies on the theme are briefly discussed.

Mishra *et al.* 2011 studied *Rosa indica* leaves, stems and flowers screened against various pathogenic bacterial strains to study the antimicrobial properties of the plant. The result of this study showed that the rose flower was found best source of antibacterial activity against the microorganisms.

Zeng *et al.* 2011 studied ailments because of the "oxidative stress" that can be removed with antioxidants. To evaluate the total phenolic, flavonoid, and Vitamin C contents antioxidant activity of plants regularly used. Cruz *et al.* 2006 studied the antimicrobial activity of crude methanol extract (CE), hexane, ethyl acetate, and butanol fractions, as well as four pure compounds obtained from *Rubus imperialis* (Rosaceae). The experiments showed that the extract and some fractions exhibited antimicrobial action, particularly against the Gram-positive bacteria tested.

Dehghan *et al.* 2010 studied the antibacterial, antioxidant, and cytotoxicity of methanolic and aqueous extracts of *Rosa hemisphaerica* Herrm. Results of this study showed it is antimicrobial only against S. aureus and toxic to the Hela cells as well as human lymphocytes. Hence, the *R. hemisphaerica* is considered important for health.

Abdel-Hameed *et al*. 2013 studied that Ward Taifi (Taif rose) is measured as one of the most important cost-effective products of Taif, Saudi Arabia. In this research both fresh and dry Taif rose were used and physiochemical investigated. The methanol extracts and its -butanol and aqueous fractions for both fresh and dry Taif rose could be used as defensive and remedial useful natural agents for diseases in which free radicals are concerned after more in vitro and in vivo studies. *Rosa brunonii* Lindl which is wildly available in different parts of Murree-Punjab Buhrban Upper Topa, Lower Topa, and Kotli Sattian Danoi and Hazara division and is used for a number of ailments. The eradicating potential of this plant for pathogenic, infectious, and syndrome-related

disorders is well established in rural peoples because of their common use. The plant bioactive compounds and their potential role as new antimicrobial leads especially in the Rosa genus have received special attention as sources of new antimicrobial agents Ren *et al.* 2003; Kashani *et al.* 2011). This prompted us to investigate *Rosa brunonii* Lindl their physicochemical attributes and biological properties available in Murree and its suburbs.

Materials and methods

Identification and collection of plant material: Fresh flowers were collected from five localities of Murree hills at an altitude ranging from 1000-2000 meters in October – December, from Lower topa (RbL), Bhurban (RbB), and Kotli Sattian (RbK), Danoi (RbD) and Gaora Gali (RbG) and brought to the laboratory of Forest Chemistry branch, Pakistan Forest Institute, Peshawar, for further processing.

The different parts of collected plants were evaluated for the average weight of rosehips, pulp/seed ratio, diameter, and length at their mature / at the end of the flowering stage. Likewise, the biochemical constituents including Vitamin C, phytochemical screening, total phenolic compounds, and flavonoids were investigated using the standard methods, as per the below procedures. i.e., Sampling, Average weight of rosehip, Pulp/seed ratio of rosehips, Diameter and length of rosehips, Estimation of Vitamin C, Preparation of Reagents, Preparation of calibration graph, Determination of L-ascorbic acid in rosehips of wild roses, Phytochemical Analysis, Preparations of standard solutions for total phenolic contents, Determination of total polyphenolic contents

Determination of Flavonoids: By using the modified version of the spectrophotometer method, the total flavonoid content in wild rose seed samples were evaluated (Sharma *et al.* 2010).

Table 1. Methodology for the determination of antimicrobial activities of *Rosa brununii* extracts

Day	Activities
Day 1	In a laminar flow hood, after sterilization, nutrient agar media was added to the plates it was incubated at 37°C for 24 hours to avoid any contagion
Day 2	The microbial stock culture was prepared again on nutrient agar plates with the help of a loop streaking (known as the first streak) was done.
Day 3	On the fresh media plates, the first streak culture was again streaked (known as the second streak), and incubated at 37°C for 24 hours.
Day 4	In the shaking water bath for 18 hours, the second streaked cultures were inoculated into the sterilized nutrient broth in flasks s at 37°C incubation was done.

Day 5	The microbial cultures from the flask were standardized in sterilized nutrient broth. Standardized microbial inoculums were seeded into the nutrient agar plates in different concentrations
Day 6	Digital camera pictures were used to measure the Zone of inhibition

Phytotoxic Activity:

Procedure: A standardized procedure (Ahmad 2010) was followed* for phytotoxicity. By counting the number of damaged plants, results were taken after seven days of incubation.

Insecticidal Activity: The insecticidal activity of the subject plant materials using the contact toxicity assay procedure was determined (Ahn *et al.,* 1995).

Procedure: In controlled humidity and temperature conditions, pests were reared in sterilized breeding media, in plastic bottles. Insects of identical size and age were selected. The number of survivals was counted by using the formula, Percentage Mortality = 100-No. of alive insects in test x 100. It indicated the number of insects alive in control Permethrin (235.9 µg/cm²) as positive and ethanol as a negative control.

Brine shrimp lethality bioassay

Procedure: Finally survived brine shrimps were observed using a magnifying glass. To examine the data Finney computer program (Probit analysis) was used and determined LD50 values with 95% confidence interval

Results and discussion

In this study, *R.brununii* was investigated for its possible biological/pharmacological potential using standard procedures. The major parameters of analysis are physiochemical factors and biological assays including antibacterial, antifungal, phytotoxic, insecticidal, and cytotoxic.

Effect of locality on Physical characteristics and Vitamin C concentration of Rosehip

Results indicated the variation of the properties among and within collection sites, which may be due to differences in altitude, climate, or nature of the soil.

Vitamin C concentration of Wild Rosehip: Samples collected from Bhurban had the highest average concentration (3000mg/100g), and a minimum from Danoi was 835 mg/100g. Higher concentration was also present in the samples from some sites of Kotli Sattian and Ghora Gali which was1156 mg/100g and 2494mg/100g respectively.

Phytochemical investigations

Screening for Flavonoids and Phenolic Compounds: Due to various groups of natural products, *Rosa brununii* was screened. The study reveals that flavonoids and phenolic contents confirm that the plant also contains flavonoids group and phenolic compounds. Results revealed that the range of total phenolic compounds and total flavonoid compounds were 0.18-1.60mg/100g and 2.44-5.76mg/100g respectively. This research has confirmed that *R. brununii* contains both total phenolic and total flavonoid contents.

Pharmacological investigations of antibacterial activity

Crude ethanolic extract: The crude ethanolic extract showed higher activity against *S. aureas* (48.43%), *P. aeruginosa* (38.09%), and moderate activity against *B. subtilis* (33.87%), *E. coli* (32.42%), *E. carotovora* (31.66%), *S. typhi* (30.88%) and *K. pneumoniae* (30.76%) in the plant extract of Kotli Sattian. In the plant extract of Lower topa crude ethanolic extract showed higher activity against *E. coli* (39.34%), *B. subtilis* (38.70%), *E. carotovora* (38.33%), moderate activity against *P. aeruginosa* (31.74%), *S. typhi* (23.52 %) and no activity against *K. pneumoniae* (0%) and *S. aureas* (0%). In the plant extract of Danoi crude ethanolic extract showed higher activity against *B. subtilis* (48.38%), *P. aeruginosa* (39.68%), *K. pneumoniae* (38.46%) and *S. aureas* (32.81%), moderate activity against *E. coli* (31.14%), and no activity against *S. typhi* (0%) and *E. carotovora*. In the plant extract of Bhurbhan the highest activity was observed against *E. coli* (39.34%), *S. aureus* (32.81%), moderate activity against *B. subtilis* (29.03), *S. typhi* (25%), *K. pneumonia* (24.61%), and no activity was against *P. auriginosa* (0%) and *E. carotovora* (0). Whereas in the plant extract of Ghora Gali crude ethanolic extract showed higher activity against *E. carotovora* (41.66%), *S. typhi* (39.70%), *B. subtilis* (38.70%), E. coli (%), and no activity against *P. auriginosa* (0%), *K. pneumoniae* (0%) and *S. aureas* (0%). Low activity against most of the test pathogens is shown in the results.

Antifungal activity

Crude methanolic extract and fractions: A study shows that Amphotericin-B and Miconazole served as standard drugs. All the test samples were dormant against *A. flavus*, *F. saloni*, Trichophyton, *C. albicans*. The results point out that the crude ethanolic extract exhibited no significant antifungal activity.

Phytotoxic activity

Crude ethanolic extract: The results of the phytotoxic activity of the test samples using Lemna assay. The oils showed moderate activity of 50 and 40% respectively. At the concentration of 1000 and 100 µg/ml, At the concentration of 10 µg/ml, the sample showed low activity of 25%.

Insecticidal assay: Crude ethanolic extract

The insecticidal activity of *Tribolium castaneum*, *Rhizopertha dominica*, and *Callosbruchus analis* was used as a model system to assess the test samples. No activity against any of the test insects is indicative that this genus has no insecticidal potential.

Brine shrimp lethality bioassay

Crude methanolic extract: Brine shrimp (Artemia salina) lethality bioassay was used to check the cytotoxic effect of the test samples. The results indicate that *Rosa brununii* showed very low toxic effects at 1000 µg/ml. Out of 30 shrimps, 27 survived. At 100 µg/ml, 28 shrimps out of 30 survived. Out of 30, 30 shrimps survived at 10 µg/ml. The LD50 value was 7.45 µg / ml. they cannot be used as a cytotoxic agent, when required as they show very low cytotoxicity in this study an effort was made to assess one of the important Specie of family Rosaceac named as *Rosa brununii* for its vital medicinal values. However, we can propose further comprehensive study is required to assess the antimicrobial evaluation of this plant.

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