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SCREENING OF SECONDARY METABOLITES AND THEIR ANTIMICROBIAL ACTIVITY IN *CENTAURIUM ERYTHRAEA*

Mohsin Ali Hassni[1]*, Laraib Zia-ul-Qamar [1], Muhammad Tariq Hasni[2], Khan Muhammad Babar[3], Abdul Aleem[4], Muhammad Din Kakar[4], Muhammad Sajid Hasni[5], M. Siraj Saif[6], Zia Ud Din[7]

[1]**Department of Pathology,** Aria Institute of Medical Sciences, Quetta, Pakistan
[2]**Department of Orthopedic,** Bolan Medical College, Quetta, Pakistan
[3]**Department of Surgery,** Bolan Medical College, Quetta, Pakistan
[4]**Department of Pathology,** Bolan Medical College, Quetta, Pakistan
[5]**Department of Nursing & Allied Health Sciences,** Alhamd Islamic University, Quetta, Pakistan
[6]**Department of Pathology,** Sheikh Mohamed Bin Zayed Al Nahyan Institute of Cardiology, Quetta, Pakistan
[7]**Center for Advanced Studies in Vaccinology and Biotechnology,** University of Balochistan, Quetta, Pakistan

***Corresponding Author:** Mohsin Ali Hassni. E. mail: mohsin.mlt18@imdcollege.edu.pk

Abstract

Centaurium erythraea, commonly known as Centaury, is found in the hot and arid regions of Balochistan, such as Turbat and other areas of Kech. Widely used as a medicinal herb in many parts of Europe, Centaury is often prepared as a tisane and is believed to offer therapeutic benefits, particularly for gastric and liver-related ailments. This plant contains a variety of secondary metabolites, including tannins, saponins, and reducing sugars, which are valuable in the medicine and pharmaceutical industries.

In this research, it was demonstrated that Centaury also exhibits antimicrobial activity. It is effective against gram-negative bacteria like E. coli, and gram-positive bacteria such as S. aureus, and shows limited activity against Bacillus species. Given its medicinal properties, Centaury holds potential for use in therapeutic industries. Furthermore, it contributes to advancements in drug development and offers insights into plant ecology and evolution

Keywords: *Centaurium erythraea, Balochistan, Evaluation, Kech, Secondary Metabolites, Therapeutic benefits*

INTRODUCTION

Humanity has relied on plants for survival throughout recorded history. Their structural qualities make them versatile, serving as a primary food source, building material, weapon, and source of medication. Each culture possesses indigenous knowledge systems passed down through generations, enabling the discovery of medicinal plants used in treatments. Researchers focus on isolating and characterizing functional compounds from medicinal herbs that can act as effective antibiotics without adverse health effects (1).

Oral health continues to be challenged by tooth decay and periodontal conditions, exacerbated by the lack of modern, effective, and safe treatment options. Amid the growing antimicrobial resistance crisis, traditional medicinal plants are gaining attention as a source of novel bioactive compounds. Nine Algerian medicinal plants (*Artemisia herba-alba, Centaurium erythraea, Juglans regia, Laurus nobilis, Matricaria recutita, Mentha pulegium, Mentha piperita, Origanum vulgare, and Taraxacum officinale*) were systematically tested for their antimicrobial and anti-biofilm properties using ethanolic and aqueous extracts (2).

This study focuses on *Centaurium erythraea*, commonly known as common centaury, a plant in the Gentianaceae family. Found across Europe, North Africa, and Southwest Asia, it is widely used as a natural medicine. Its applications include enhancing gastrointestinal motility, preventing stomach damage, and reducing total blood cholesterol levels. In China, secoiridoid extracted from this plant has been approved as a novel drug for treating chronic active hepatitis and acute jaundice. Traditionally, *C. erythraea* has been

used to treat digestive issues, anemia, and various other ailments, thanks to its abundance of secoiridoid glucosides and xanthones (3).

The Gentianaceae family comprises 99 genera and over 1,736 species, distributed worldwide. These plants include annual and perennial plants, including small trees, shrubs, and herbs, with significant pharmaceutical and horticultural importance. Many species produce bitter iridoids, xanthones, flavonoids, and other metabolites used in medicine and flavoring. Other species are cultivated for their ornamental flowers, showcasing diverse colors and patterns. The leaves, either sessile or petiolate, vary in size and shape, further enhancing their aesthetic value (4).

In vitro morphogenetic studies have focused on seven genera of the Gentianaceae family, including Centaurium, Eustoma, Exacum, Gentiana, and Swertia. However, somatic embryogenesis (SE) processes have been documented in only a few species. Bareová and Kaminek (1984) provided the first SE report for the Gentianaceae family, emphasizing Centaurium erythraea (5).

In somatic embryogenesis (SE) and shoot organogenesis (SO), arabinogalactan proteins (AGPs) play a crucial role. Mechanical injury during in vitro treatments triggers dedifferentiation, cell cycle reactivation, vascular recovery, and defense responses. This injury-induced signaling can promote SE and SO. However, the contribution of AGPs to these processes in centaury has not been extensively explored (6).

The antioxidant activity of *C. erythraea* explains its gastro-protective effects observed with aqueous-ethanol extracts. Cellular oxidation pathways produce reactive nitrogen and oxygen species that can damage DNA, proteins, and lipids. Antioxidants in C. erythraea neutralize these radicals, preventing cell damage and addressing oxidative stress-related disorders (7).

Despite the potential of *C. erythraea* as a model organism for developmental biology, molecular studies have been limited by the lack of genetic sequence data. Recent advancements, including the ragp bioinformatics workflow and transcriptome resources, have enabled a comprehensive analysis of the *C. erythraea* AGP family. This research identified AGPs implicated in SE, SO, and wound responses. Among the 18 identified AGP genes, subclasses such as fasciclin-like AGPs (FLAs), receptor kinase-like AGPs (KLAs), and AG peptides (AGps) were explored. FLAs, known for their role in zygote development in A. thaliana and other species, were further studied for their involvement in *C. erythraea* SE. Newly identified KLAs, a novel AGP class, represent promising signaling molecules (8).

Temporal expression patterns of the selected 18 genes were analyzed after mechanical wounding of leaf explants and during SE and SO stages. Comparative analysis included samples from wild and in vitro-grown plants. The study discusses the structure, phylogenetic relationships, and potential functions of these AGPs, shedding light on their role in plant morphogenesis and response to injury.

MATERIALS AND METHODS

SAMPLE PREPARATION

For the preparation of plant extracts, 2.50 g of leaf material was weighed using a precision balance and transferred into a clean beaker. Ethanol (50 mL) was measured using a graduated cylinder and added to the beaker containing the leaf material. The mixture was stirred thoroughly using a glass rod and subsequently covered with aluminum foil. The same procedure was repeated for the stem, flowers, and whole plant extracts. For methanol extraction, 2.50 g of each plant part (leaves, stems, flowers, and whole plant) was processed similarly, substituting ethanol with methanol. Distilled water extracts were prepared following the same protocol, using distilled water as the solvent.

FILTRATION

After extraction, falcon tubes were labeled to match the corresponding beakers. The contents of each beaker were filtered through filter paper, and the solid residues were discarded. The resulting filtrates were transferred to the labeled falcon tubes to minimize evaporatio3n. The tubes were initially covered with plastic wrap before sealing tightly with caps. All tubes were stored at 4°C in a refrigerator until further analysis.

PHYTOCHEMICAL SCREENING

ALKALOID TEST

Mayer's reagent was prepared by dissolving 1.36 g of mercuric chloride and 5 g of potassium iodide. To 1 ml of each sample, 6–8 drops of Mayer's reagent were added. The formation of a precipitate indicated the presence of alkaloids.

FLAVONOID TEST

A few drops of dilute hydrochloric acid and dilute sodium hydroxide were added to 1 mL of each sample. A color change indicated the presence of flavonoids.

TANNIN TEST

To 1 mL of each sample, a few drops of ferric chloride were added. The appearance of a brownish color indicated the presence of tannins.

TERPENOID TEST

To 2.5 mL of each sample, 1.5 mL of sulfuric acid and 1 mL of chloroform were added. A color change indicated the presence of terpenoids.

LIPID TEST

To 500 µL of each sample, 0.5 mL of 50% ethanol and 1 mL of 20% sodium hydroxide were added. The mixture was stirred and heated in a water bath for 15 minutes, followed by the addition of 1 g of crystalline sodium hydroxide and further heating for 3 minutes. The formation of foam indicated the presence of lipids.

SAPONIN TEST

To 1 mL of each sample, 19 mL of distilled water was added, and the mixture was shaken vigorously. The formation of a haze indicated the presence of saponins.

STEROID TEST

To 500 µL of each sample, 1 mL of chloroform and 6 drops of concentrated hydrochloric acid were added. The formation of a red ring indicated the presence of steroids.

CARBOHYDRATE TEST

To 1 mL of Fehling's solution in each of the 12 test tubes, the tubes were placed in a water bath and boiled for 1 minute. Subsequently, 1 mL of each sample was added, and the tubes were boiled again for 5 minutes. A color change indicated the presence of carbohydrates.

ALDEHYDE TEST

To each of the 12 test tubes, 12 drops of silver nitrate and 6 drops of dilute sodium hydroxide were added, followed by 6 drops of dilute ammonium solution. The mixtures were shaken for a few seconds, and 6 drops of each sample were added. The tubes were then boiled in a water bath for 5 minutes. The appearance of a reaction indicated the presence of aldehydes.

RESULTS AND DISCUSSION

PHYTOCHEMICAL INVESTIGATION

The primary objective of this study was to investigate the secondary metabolites and antimicrobial properties of *Centaurium erythraea*, a medicinal plant indigenous to the southern Balochistan region (Turbat). The results demonstrated that the plant contains a variety of secondary metabolites, including reducing sugars, tannins, saponins, steroids, and terpenoids, as detailed in Table I. Among these, tannins play a significant role due to their hydroxyl groups, which are responsible for their scavenging effects (9). These metabolites are critical for plant defense and have broad pharmaceutical applications. However, flavonoids,

reported in previous studies as potent antioxidants, were not detected in this investigation. Methanolic extracts of *C. erythraea* have been previously reported to protect diabetic rat kidneys and livers and mitigate oxidative damage to DNA and lipids.

Table I. The results of different phytochemical tests used to screen secondary metabolites in Centaurium erythraea

S. No.	Secondary Metabolites	Result
1	Carbohydrates Test (Reducing Sugar)	Positive
2	Carbohydrates Test (Non-Reducing Sugar)	Negative
3	Flavonoid	Negative
4	Alkaloid	Negative
5	Tannin (Ferric Chloride test)	Positive
6	Tannin (Gelatin test)	Positive
7	Lipids	Negative
8	Saponin	Positive
9	Steroid	Positive
10	Terpenoids	Positive
11	Aldehyde	Negative

Interestingly, alkaloids were also absent in this study, although earlier research identified them in the form of gentianine (a xanthone alkaloid), swertiamarin (a xanthone alkaloid), sweroside (a monoterpenoid indole alkaloid), and secoiridoids. These alkaloids are known to have potential pharmacological applications, including antimicrobial activity (10). The antimicrobial properties of various plant extracts were evaluated in this study and are represented in the graphs provided.

ANTIMICROBIAL ACTIVITY AGAINST *LACTOBACILLUS PARACASEI* A13

The ethanolic extract of the flower exhibited the highest antimicrobial activity (7%) against *Lactobacillus paracasei* A13, followed by the methanolic extract of the flower (5%). The stem extracts, both methanolic and ethanolic, showed minimal activity (2%), while the ethanolic extract of the leaves displayed the lowest activity (1%). In contrast, the methanolic extract of the leaves exhibited 6% activity. Whole plant extracts showed minimal activity, with both methanolic and ethanolic extracts displaying only 1% activity. Distilled water extracts of all plant parts exhibited no activity (Fig. 1). When compared to the standard drug used as a positive control, only the ethanolic flower extract showed comparable high activity, while the distilled water used as a negative control exhibited no activity (11).

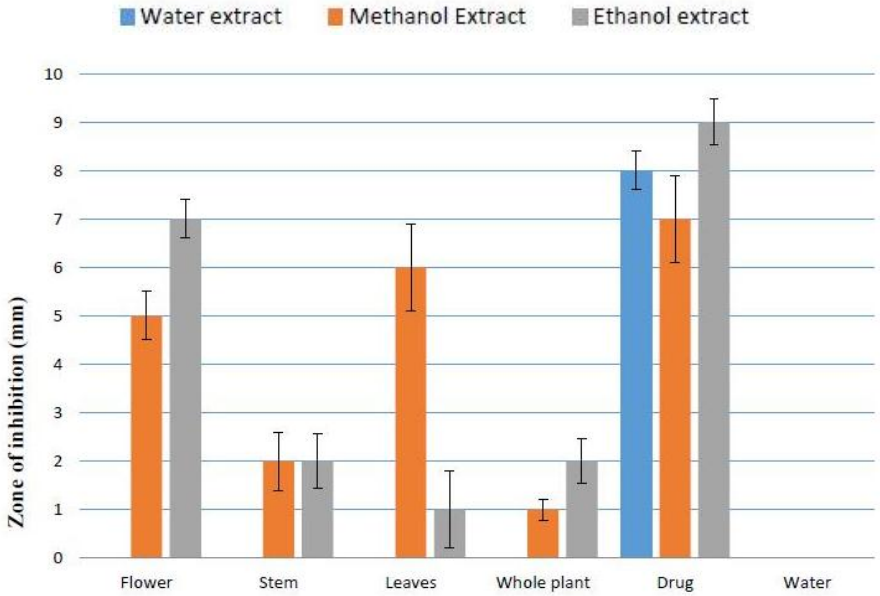

Fig. 1. Bioactive assay of *C. erythraea* against Gram- positive bacteria *Lactobacillusparacasei* A13

ANTIMICROBIAL ACTIVITY AGAINST *LACTOBACILLUS CASEI* A14

The antimicrobial activity of *C. erythraea* extracts against *Lactobacillus casei* A14 revealed that the ethanolic and methanolic flower extracts both exhibited 5% activity. Ethanolic extracts of the stem showed higher activity (6%) than their methanolic counterparts (3%). Methanolic and ethanolic leaf extracts both demonstrated 3% activity, while the methanolic whole-plant extract exhibited 5% activity (Fig. 2). The ethanolic whole-plant extract demonstrated higher activity at 7%, comparable to the standard drug (9%). All distilled water extracts and the negative control showed no activity (12, 13).

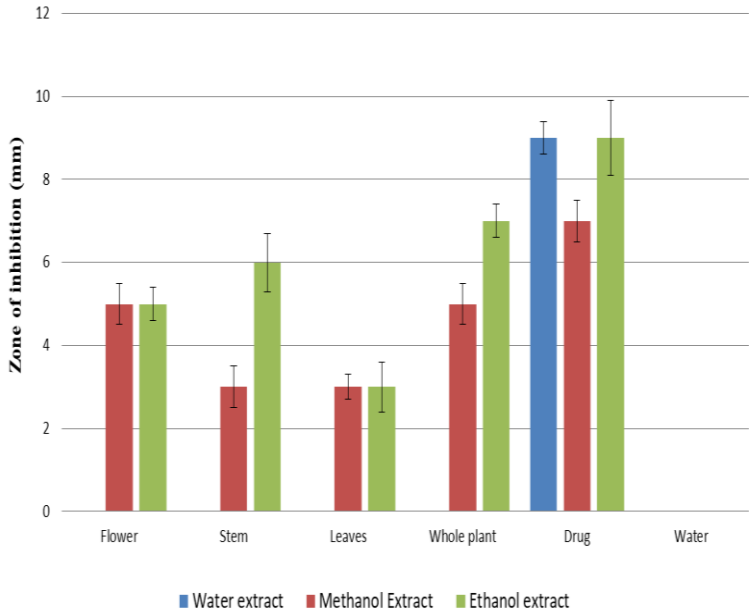

Fig. 2. Bioactive assay of *C. erythraea* against *Lactobacillus casei* A14

ANTIMICROBIAL ACTIVITY AGAINST *E. COLI*

The antimicrobial activity against *E. coli* showed promising results. Methanolic flower extracts exhibited the highest activity (9%), followed by ethanolic flower extracts (7%) and distilled water flower extracts (5%). Stem extracts also demonstrated significant activity, with methanolic extracts at 11% and ethanolic extracts at 8%. Leaf extracts showed moderate activity; ethanolic extracts exhibited 8% activity, while distilled water and methanolic extracts exhibited 5%. The whole-plant methanolic extract displayed 9% activity, while the ethanolic extract demonstrated the highest activity at 13% (Fig. 3). Compared to the standard drug, the ethanolic whole-plant extract surpassed the standard drug's activity, highlighting its potential as a viable alternative (14).

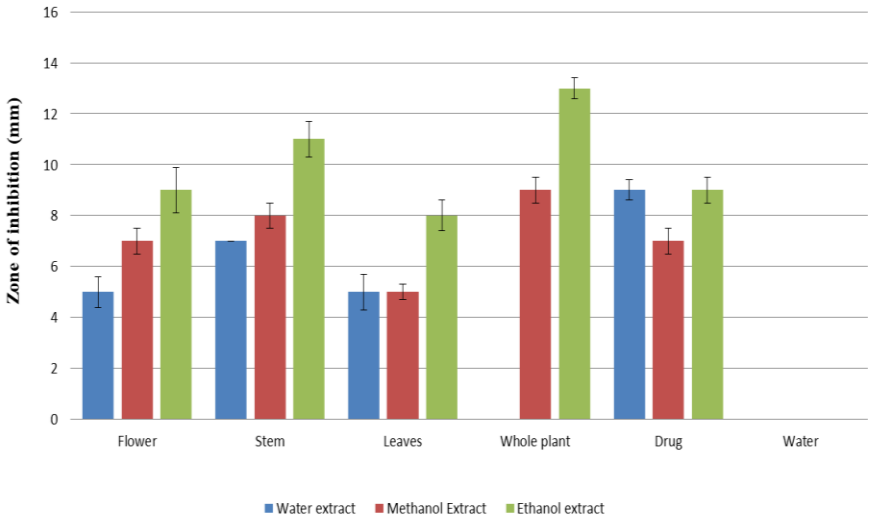

Fig. 3. Bioactive assay of *C. erythraea* against Gram *E. coli*

ANTIMICROBIAL ACTIVITY AGAINST *S. AUREUS*

The extracts of *C. erythraea* also exhibited activity against *S. aureus*. Distilled water extracts of flowers showed 3% activity, methanolic extracts 5%, and ethanolic extracts 7%. Stem extracts exhibited higher activity, with distilled water extracts at 7%, methanolic extracts at 9%, and ethanolic extracts at 10%. Whole-plant extracts demonstrated significant antimicrobial properties, with methanolic and ethanolic extracts showing 9% and 11% activity, respectively. The ethanolic whole-plant extract exhibited superior activity (11%) compared to the standard drug (15).

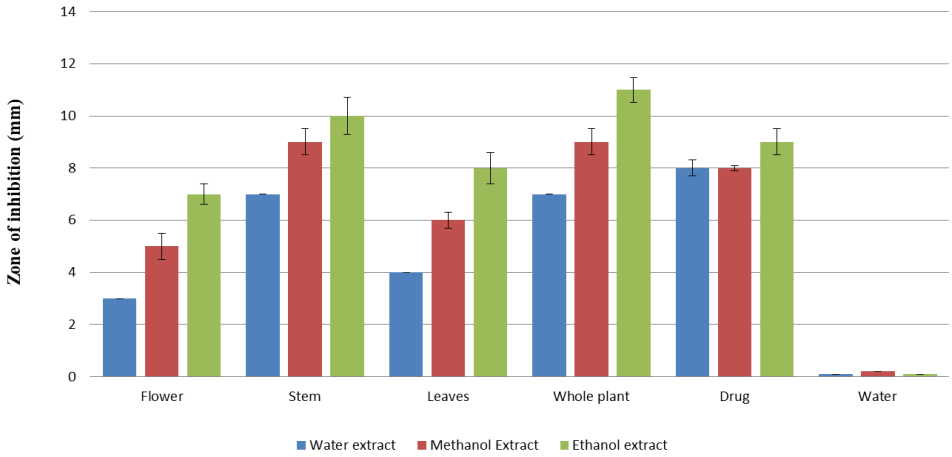

Fig. 4. Bioactive assay of *C. erythaea* against *S. aureus*

The absence of activity in distilled water extracts and the negative control validates the antimicrobial potential of the ethanolic and methanolic extracts. The minimal activity observed in some water extracts may be due to contamination or sterilization issues.

This study highlights the potential of *C. erythraea* as a source of bioactive compounds with antimicrobial properties. The presence of secondary metabolites such as tannins, saponins, and terpenoids further supports its traditional medicinal use. The differential antimicrobial activities observed across various extracts suggest that the solvent used for extraction plays a crucial role in isolating bioactive compounds. The ethanolic extracts, in particular, demonstrated superior antimicrobial activity, making them a promising candidate for further investigation in drug development.

Future research could focus on the isolation and structural elucidation of individual bioactive compounds from *C. erythraea* and their mechanism of action against various microbial strains. Additionally, studies exploring the synergistic effects of these compounds and their potential application in pharmaceutical formulations could significantly enhance our understanding of the plant's therapeutic potential.

CONCLUSION

The phytochemical analysis revealed that the ethanolic extract of the whole plant of *Centaurium erythraea* exhibited significantly higher effectiveness against Gram-negative bacteria, such as *E. coli* and *S. aureus*, compared to the extracts derived from individual plant parts (flower, stem, and leaf) as well as water and methanolic extracts. The ethanolic extract demonstrated remarkable antimicrobial activity, particularly against *E. coli*. In contrast, limited efficacy was observed against *Lactobacillus* strains, which may be attributed to their generally non-pathogenic nature, except in rare cases. Additionally, the pronounced activity of *C. erythraea* against Gram-negative bacteria may reflect its inherent specificity towards these bacterial types.

Limitations:

This study was conducted with limited resources; however, more rigorous assays or in vivo testing could help identify additional bacterial strains and explore potential synergistic effects with established antibiotics.

Conflict of Interest:

The authors have no conflict of interest.

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