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Antimicrobial Activity of Catharanthus Roseus Root Extract against Neisseria gonorrhoeae: In Vitro Study of Maceration and Percolation Extraction Methods

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ABSTRACT:

Background: Neisseria gonorrhoeae is the causative agent of gonorrhea, a sexually transmitted infection that can cause severe complications including gonococcal conjunctivitis. The increasing antibiotic resistance of N. gonorrhoeae has necessitated the search for alternative antimicrobial agents. Catharanthus roseus (Tapak Dara) is a medicinal plant known for its various pharmacological properties including antimicrobial, anti-inflammatory, and antioxidant effects. This study aimed to evaluate the antimicrobial activity of C. roseus root extract against N. gonorrhoeae using maceration and percolation extraction methods. This in vitro study evaluated the antimicrobial activity of C. roseus root extract against N. gonorrhoeae using two extraction methods: maceration and percolation. The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Turbidity was assessed based on optical density (OD) measurements. Zone of inhibition was measured using the disc diffusion method. Colony counts were performed using the pour plate method. For the maceration extract, the MIC was determined at 30% concentration and the MBC at 70% concentration. The percolation extract showed higher antimicrobial activity with MIC at 40% and MBC at 70%. Zone of inhibition measurements demonstrated dose-dependent activity, with the maceration extract showing mean

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inhibition zones ranging from 10.56 mm (10%) to 37.81 mm (100%), while percolation extract showed zones from 12.63 mm (20%) to 36.31 mm (100%). Colony counts decreased progressively with increasing extract concentrations in both extraction methods. Catharanthus roseus root extract demonstrates significant antimicrobial activity against N. gonorrhoeae. The maceration method showed better antimicrobial efficacy with lower MIC values compared to percolation. These findings support the potential use of C. roseus root extract as an adjuvant therapy for gonococcal infections, including conjunctivitis.

INTRODUCTION

Neisseria gonorrhoeae is a Gram-negative diplococcus and the causative agent of gonorrhea, one of the most common sexually transmitted infections worldwide. In 2020, approximately 677,769 cases of gonorrhea were reported to the Centers for Disease Control and Prevention (CDC), making it the second most reported sexually transmitted infection in the United States [1]. The reported rates have increased by 111% since 2009, representing a significant public health concern. In Indonesia, studies have shown increasing prevalence of gonorrhea, with 22.4% of asymptomatic patients testing positive for the infection [2].

Gonococcal conjunctivitis is a severe ocular infection caused by N. gonorrhoeae, characterized by hyperacute conjunctival injection, chemosis, and copious purulent discharge [3]. This condition is particularly concerning because N. gonorrhoeae can rapidly invade the cornea, potentially causing corneal perforation and blindness within hours if left untreated [4]. The condition affects both neonates (ophthalmia neonatorum) and adults, with adult cases typically resulting from autoinoculation or exposure to infected genital secretions [5].

Clinically, gonococcal conjunctivitis presents with bilateral hyperacute conjunctival inflammation, marked chemosis, and profuse purulent discharge that reaccumulates rapidly after cleaning [6]. Corneal involvement can lead to epithelial defects, stromal infiltrates, and rapid perforation if not treated promptly.[7] Several case reports have documented the severity of this condition, including corneal perforation requiring emergency intervention [8][9].

The treatment of N. gonorrhoeae infections has become increasingly challenging due to the emergence of antibiotic-resistant strains. Over the past 80 years, N. gonorrhoeae has developed resistance to sulfonamides, penicillin, tetracycline, ciprofloxacin, azithromycin, and most recently, ceftriaxone [10]. This rising resistance has led the World Health Organization to classify N. gonorrhoeae as a high-priority pathogen in the global priority list of antibiotic-resistant bacteria [11].

The mechanisms of antimicrobial resistance in N. gonorrhoeae include chromosomal mutations and acquisition of resistance genes through horizontal gene transfer [12]. Recent studies have documented the emergence of extensively drug-resistant strains that are resistant to multiple classes of antibiotics, including extended spectrum cephalosporins [13]. This situation has created an urgent need for alternative therapeutic approaches.

In the context of increasing antibiotic resistance, there has been growing interest in traditional medicine and natural products as alternative sources of antimicrobial agents [14]. Herbal eye drops have been used traditionally for treating various ocular conditions, including inflammatory and infectious diseases [15]. Several studies have reported the effectiveness of herbal formulations in managing ocular surface diseases with minimal side effects.

Catharanthus roseus (L.) G. Don, commonly known as Madagascar periwinkle or Tapak Dara in Indonesia, is a medicinal plant belonging to the Apocynaceae family [16]. This plant has been traditionally used in various cultures for treating diabetes, hypertension, and various infections [17]. In South Africa, traditional healers have used C. roseus root preparations for treating gonorrhea and other sexually transmitted infections [18].

Modern pharmacological studies have demonstrated that C. roseus possesses antimicrobial, anti-inflammatory, antioxidant, and anticancer properties.[19] The plant contains over 400 alkaloids, including vinblastine and vincristine, which are important anticancer agents used clinically.[20] Other important alkaloids include ajmalicine, serpentine, vindoline, and catharanthine, which are distributed in different parts of the plant [21].

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The antimicrobial activity of C. roseus has been attributed to its rich content of alkaloids, flavonoids, saponins, and phenolic compounds [22]. Hanafy et al. demonstrated that methanolic root extract of C. roseus showed significant antimicrobial activity against various microorganisms including Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa [23]. The activity was found to increase with increasing extract concentration, indicating a dose-dependent effect.

The antibacterial mechanism of C. roseus extract involves multiple targets including disruption of bacterial cell wall synthesis by interfering with peptidoglycan components, protein denaturation, membrane stability disruption, and inhibition of nucleic acid synthesis [24]. Recent studies have shown that C. roseus can counteract antibiotic resistance in methicillin-resistant Staphylococcus aureus (MRSA) by downregulating PBP2a synthesis [25]. Furthermore, combination of C. roseus extract with conventional antibiotics has shown synergistic effects [26].

Although previous studies have demonstrated antimicrobial activity against various microorganisms, studies specifically evaluating the activity of C. roseus against N. gonorrhoeae are limited [27]. Given the urgent need for alternative treatments for gonococcal infections and the traditional use of C. roseus for treating sexually transmitted infections, this study was conducted to evaluate the antimicrobial activity of C. roseus root extract against N. gonorrhoeae using two different extraction methods: maceration and percolation.

METHODS

Study Design

This was an in vitro experimental study conducted to evaluate the antimicrobial activity of C. roseus root extract against N. gonorrhoeae. The study compared two extraction methods: maceration and percolation. The experimental design followed the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) for antimicrobial susceptibility testing.

Plant Material and Extraction

Catharanthus roseus roots were collected and identified at the Faculty of Pharmacy, Universitas Sumatera Utara. The plant material was authenticated by a taxonomist, and a voucher specimen was deposited at the herbarium. Two extraction methods were employed:

Maceration: The dried root powder was macerated using 75% ethanol as the solvent. Remaceration was performed twice. The filtrate was concentrated using a rotary evaporator and then dried in an oven at 50°C to ensure complete solvent evaporation.

Percolation: The dried root powder was subjected to percolation using 75% ethanol. The percolate was collected and concentrated using similar methods as maceration.

Bacterial Strain

Neisseria gonorrhoeae was obtained from the Department of Microbiology, Faculty of Medicine, Universitas Sumatera Utara. The bacteria were cultured on GC agar (Oxoid) supplemented with growth factors and incubated at 37°C with 5% CO2 for 24-48 hours. Bacterial suspensions were prepared at 0.5 McFarland standard (1.5×10^8 CFU/mL) for antimicrobial testing.

Microdilution Assay

The microdilution method was used to determine the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC). Serial dilutions of the extract were prepared at concentrations of 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, and 100%. Each well was inoculated with bacterial suspension and incubated at 37°C with 5% CO2 for 24 hours. Turbidity was assessed based on optical density (OD) measurements. The MIC was defined as the lowest concentration showing no visible turbidity. For MBC determination, samples from clear wells were subcultured onto fresh GC agar plates.

Disc Diffusion Assay

The disc diffusion method (Kirby-Bauer) was used to measure the zone of inhibition. Bacterial suspensions (0.5 McFarland) were evenly inoculated onto GC agar plates. Paper discs containing different concentrations of extract were placed on the agar surface. Plates were incubated at 37°C with 5% CO2 for 24 hours. The diameter of the inhibition zone was measured in millimeters using a caliper.

Colony Count

Colony counts were performed using the pour plate method. Serial dilutions (10^{-5} to 10^{-7}) were prepared from bacterial suspensions. Aliquots (0.1 mL) from each dilution were plated onto Mueller-Hinton agar and incubated at 37°C for 24-48 hours. Colonies were counted using a colony counter, and results were expressed as CFU/mL.

Statistical Analysis

Data was analyzed using descriptive statistics. Mean values and standard deviations were calculated for zone of inhibition measurements and colony counts. All experiments were performed in quadruplicate to ensure reproducibility of results.

RESULTS AND DISCUSSION

Maceration Extract Results

The microdilution assay of the maceration extract demonstrated concentration-dependent antimicrobial activity against N. gonorrhoeae. The results are summarized in Table 1.

Table 1. Turbidity Assessment of Maceration Extract Based on Optical Density

Concentration	Replicate 1	Replicate 2	Replicate 3	Replicate 4
10%	High	High	High	High
20%	High	High	High	High
30%	Low	Low	Low	Low
40%	Low	Low	Low	Low
50%	Low	Low	Low	Low
60%	Low	Low	Low	Low
70%	Low	Low	Low	Low
80%	Low	Low	Low	Low
90%	Low	Low	Low	Low
100%	Low	Low	Low	Low
Control (-)	High	High	High	High
Control Media	None	None	None	None

Based on the microdilution results, the Minimum Inhibitory Concentration (MIC) of the maceration extract was determined to be 30%, where visible turbidity was no longer observed. The Minimum Bactericidal Concentration (MBC) was determined at 70%, where no bacterial growth was observed upon subculture.

Table 2. Colony Count Results for Maceration Extract (CFU/mL)

Concentration	R1	R2	R3	Mean
10%	>300	>300	>300	>300
20%	>300	>300	>300	>300
30%	141	133	136	134.25
40%	127	78	82	75.25

50%	69	41	48	39.75
60%	72	19	23	21.25
70%	39	10	7	8
80%	31	0	0	0
90%	26	0	0	0
100%	17	0	0	0
Control (-)	>300	>300	>300	>300
Control Media	0	0	0	0

Percolation Extract Results

The percolation extract showed different antimicrobial activity patterns compared to maceration. The results are presented in Table 3.

Table 3. Turbidity Assessment of Percolation Extract Based on Optical Density

Concentration	Replicate 1	Replicate 2	Replicate 3	Replicate 4
10%	High	High	High	High
20%	High	High	High	High
30%	High	High	High	High
40%	High	High	High	High
50%	Low	Low	Low	Low
60%	Low	Low	Low	Low
70%	Low	Low	Low	Low
80%	Low	Low	Low	Low
90%	Low	Low	Low	Low
100%	Low	Low	Low	Low
Control (-)	High	High	High	High
Control Media	None	None	None	None

For the percolation extract, the MIC was determined at 40% concentration, while the MBC was at 70% concentration. The higher MIC observed with percolation extract suggests that this extraction method may be less efficient in extracting antimicrobial compounds compared to maceration.

Table 4. Colony Count Results for Percolation Extract (CFU/mL)

Concentration	R1	R2	R3	Mean
10%	>300	>300	>300	>300
20%	>300	>300	>300	>300

30%	>300	>300	>300	>300
40%	282	255	238	260.5
50%	267	116	107	110.75
60%	122	57	42	43
70%	98	14	21	16.75
80%	36	9	6	7.5
90%	37	0	0	0
100%	13	0	0	0
Control (-)	>300	>300	>300	>300
Control Media	0	0	0	0

Zone of Inhibition

The disc diffusion assay demonstrated dose-dependent inhibition zones for both extraction methods. The results are shown in Tables 5 and 6.

Table 5. Zone of Inhibition for Maceration Extract (mm)

Concentration	R1	R2	R3	R4	Mean
10%	10.5	10.5	10.25	11	10.56
20%	11.7	12	12	12	11.93
30%	14.25	15	14.75	14.25	14.56
40%	24	23.5	24	23	23.63
50%	26	25	25.5	25.25	25.44
60%	28.5	27	27.25	27.5	27.56
70%	30	30	30	30.5	30.13
80%	31.75	32.5	32.5	33	32.44
90%	35.75	35.5	35.25	35	35.38
100%	38	37	38	38.25	37.81
Control (-)	0	0	0	0	0

Table 6. Zone of Inhibition for Percolation Extract (mm)

Concentration	R1	R2	R3	R4	Mean
10%	0	0	0	0	0
20%	12.25	12.75	12.75	12.75	12.63
30%	15.75	19	15.75	17	16.88

40%	19.75	19.5	19	20	19.56
50%	24.5	24	24.75	24.5	24.44
60%	27	28.5	26.5	26.5	27.13
70%	30	30.5	30	28.5	29.75
80%	31.5	32	32.25	32	31.94
90%	33.5	33.5	33.5	33	33.38
100%	36.5	35.75	36.5	36.5	36.31
Control (-)	0	0	0	0	0

The maceration extract showed mean inhibition zones ranging from 10.56 mm at 10% concentration to 37.81 mm at 100% concentration. The percolation extract showed zones from 12.63 mm at 20% concentration to 36.31 mm at 100% concentration. Both extracts demonstrated progressive increase in inhibition zone diameter with increasing concentration, confirming the dose-dependent nature of the antimicrobial effect.

DISCUSSION

This study evaluated the antimicrobial activity of Catharanthus roseus root extract against Neisseria gonorrhoeae using two different extraction methods. The results demonstrated that both maceration and percolation extracts possess significant antimicrobial activity against this important pathogen, supporting the traditional use of C. roseus for treating infections [18].

The maceration extract showed a MIC of 30% and MBC of 70%, indicating effective inhibition and bactericidal activity at relatively low concentrations. The percolation extract showed slightly higher MIC at 40%, suggesting that the maceration method may be more effective in extracting antimicrobial compounds from C. roseus roots. This difference could be attributed to the longer contact time between the plant material and solvent in the maceration process, allowing for more complete extraction of bioactive compounds including alkaloids and phenolic compounds [19][21].

The antimicrobial activity observed in this study is consistent with previous reports on C. roseus. Hanafy et al. demonstrated that methanolic root extract of C. roseus showed significant antimicrobial activity against various microorganisms including Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa [23]. The activity was found to increase with increasing extract concentration, which aligns with our findings. Similarly, Naz et al. reported antimicrobial activity of C. roseus extracts against multiple bacterial and fungal pathogens [27].

The mechanism of antibacterial action of C. roseus extract is believed to involve multiple targets. Alkaloids present in the extract can disrupt bacterial cell wall synthesis by interfering with peptidoglycan components, like the mechanism described for other plant alkaloids [24]. Flavonoids and phenolic compounds contribute to antimicrobial activity through their ability to denature bacterial proteins and disrupt membrane stability. Additionally, these compounds can inhibit nucleic acid synthesis, leading to DNA damage and bacterial growth inhibition [22].

The zone of inhibition measurements demonstrated dose-dependent activity for both extraction methods. The progressive increase in inhibition zone diameter with increasing concentration confirms the concentration-dependent nature of the antimicrobial effect. This is particularly important for potential therapeutic applications, as it suggests that higher concentrations would provide greater antimicrobial efficacy. The inhibition zones observed in our study are comparable to those reported for other medicinal plants with known antimicrobial activity [28].

The colony count further support the antimicrobial activity of C. roseus extract. The progressive decrease in colony-forming units with increasing extract concentration demonstrates the bactericidal effect of the extract. At concentrations above the MBC, complete elimination of viable bacteria was observed, indicating that the extract can kill N. gonorrhoeae rather than merely inhibiting its growth.

The findings of this study have important implications for the development of alternative treatments for gonococcal infections. Given the increasing antibiotic resistance of N. gonorrhoeae, as documented by multiple surveillance studies, natural products such as C. roseus extract may serve as valuable adjuvant therapies or alternative treatments. The extract's additional anti-inflammatory and antioxidant properties may also contribute to improved clinical outcomes in gonococcal conjunctivitis [19][29].

The anti-inflammatory properties of C. roseus are particularly relevant for treating gonococcal conjunctivitis, where the inflammatory response contributes significantly to tissue damage. Studies have shown that C. roseus extracts can modulate cytokine production and reduce inflammation [29]. This dual antimicrobial and anti-inflammatory action makes C. roseus an attractive candidate for topical application in ocular infections.

Further studies are needed to identify the specific active compounds responsible for the antimicrobial activity and to evaluate the safety and efficacy of C. roseus extract in vivo. The identification of bioactive compounds through phytochemical analysis would enable standardization of the extract and quality control in future therapeutic applications [21]. Clinical trials would be necessary to establish appropriate dosing regimens and to confirm the therapeutic potential of this natural product for treating gonococcal infections.

CONCLUSION

This study demonstrates that Catharanthus roseus root extract possesses significant antimicrobial activity against Neisseria gonorrhoeae. The maceration extract showed superior activity with a MIC of 30% and MBC of 70%, compared to the percolation extract with MIC of 40% and MBC of 70%. Both extracts demonstrated dose-dependent inhibition zones and progressive reduction in bacterial colony counts.

These findings support the traditional use of roseus for treating infections and provide scientific evidence for its potential use as an adjuvant therapy for gonococcal infections, including conjunctivitis. The extract's antimicrobial properties, combined with its known anti-inflammatory and antioxidant effects, make it a promising candidate for further development as an alternative or complementary treatment option in the context of increasing antibiotic resistance.

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