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Evaluation of Antibacterial Property of *Colchicum luetum* L. Medicinal Plant of North Western Himalaya

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Abstract

The current study examines the antimicrobial potential of aqueous and ethanolic extracts derived from Colchicum luetum Baker, medicinal plant with numerous medicinal effects. The bacteria's selected viz. Staphylococcus aureus, Pseudomonas aeruginosa and Escherichia coli. Five differential combinations i.e., (10, 30, 50, 80 and 100 mg ml-1) about the two aqueous and ethanolic extracts of Colchicum luetum act used in contrast to the mentioned above bacterial strains. Ethanolic extract demonstrate significantly high antibacterial action across Pseudomonas aeruginosa, Staphylococcus aureus also Escherichia coli; alongside area about resistance (14±0.27 mm), (20±0.27 mm) and (17±0.14 mm) by 100 mg/ml. The research windup certain herb grab innovative admixture amidst important antibacterial properties. Desolation along with characterizing about these innovative compounds keep administer strong animicable worker, thus counteracting disease causing organisms. The findings indicate the potential use of extracts from the plant to develop new organic antibacterial products. It's corms and seeds could be a natural source for the pharmaceutical industry Therefore, pharmaceutical Industries can manufacture the drugs against these bacterial diseases.

Keywords: Antimicrobial, *Colchicum luetum*; Zone of Inhibition; Aqueous and Ethanolic; Himaliya

Introduction

Medicinal herbs abide generally used in medicinal production as manufactory about loaded antibiotics also appropriated in the design of fresh herbs by local population [1-5]. Herbal medicines also treated as medium as well as equitable sources for better and healthy life. These herbs having large curative significance [6-9]. Broad diversity of commonly employed antibiotics gathered together in large quantity in natural left out part of management [10-12]. This is very important to collect these resources with experience as well as responsibility to manage its continuing existence. Due to anthropogenic activities, thus these beneficial sources become scarce on earth. Worldwide there are about 10,000 herbaceous (medicinal) plant species which are venerable, due to various anthropogenic and natural activities on endangered Medicinal Plant Species of North Western Himalaya [12-19].

"*Colchicum luteum* Baker, generally called as (Suranjan-e-Talkh, Urdu) [20]". The genus Colchicum comes under family Liliaceae or Colchicaceae. Their "species" account continuously change about (100) types of species of Colchicum are dispersed unevenly across the world [21]. In the colchicum, "31picular types alkaloids has been confined" [22]. Important compound colchicine obtained from Colchicum luetum, which helps to cure against "Behcet's syndrome" [23,24]. Alkaloid "Colchicine" is the major chemical compound derived from "Colchicum genera", [25-29]. Alzheimer disease (AD) is cured by the drugs antiinflammatory obtained from plant corms of *C. luteum* [21, 30-32]. The species is facing various biotic stresses like uncontrolled grazing, illegal harvesting, deforestation, loss of habitat, construction of roads and tourist impacts etc., [19,33].

Human population is mostly get effected by contagious microbial population from old times and memorable till date. Microbial species viz. "*Pseudomonas aeruginosa*, Klebsiella pneumonia, and *Escherichia coli*" causing dermis infection, septic infection, upper as well as respiratory infection [34-37]. *Escherichia coli* cause genealogical diseases and infections. "*Staphylococcus aureus* causes intra intestinal infection, bone as well as joint effects, below respiration infection and dermis diseases" [38-40]. Thus herbal plant species were the earlier targeted weapon employed humans to cure pathogenic diseases .The Inauguration of antibiotics came in the existence in (20th) century [41,42]. Thus, in that surely to flourish different ant infectious medicines to cure various infection causes illness, first path be about to cover regional "medicinal plant her for potentional antimicrobial properties". These herbal ingredients persist an essential natural recourses to fight against severe illness disease, through worldwide [43-46]. World Health Organization WHO 1993 reported (80%) world's human life mainly vulnerable on the conventional medicines as well as main item of the "traditional therapies" associate to apply the these herbal extract either their effective/active compounds. Still an experimental

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scientific investigation of plant to resolve their "antimicrobial effective constituents is a comparatively advanced field" [47].

Material and Methods

Collection and identification

Corms of the plant *Colchicum luteum* and samplings gathered from the selected areas of Kashmir Himalaya in the onset set of March -April (2017-2018), and the identification were done at Division of Environmental Sciences, SKUAST-K Shalimar, Srinagar and verified by Dr. Haleema Bano (Assistant Professor Skuast-k). The corm plant samples were "rightly labeled and noted with the date of collection and preserved."

Preparation of extracts

The corms of plant (*Colchicum luteum*) were shade dried at (28±3°C). The preserved corms were prepared into fine pulverized by applying pestle and mortar, extracted using ethanol as well as water as "solvents". The solvent extracts so obtained were squeezed through four folds cloth of (muslin) and lastly using "whatman filter paper (No.1)" for complete filtration. All the filters were centrifuged at 5000 rpm for 20 minutes. Concentrated solid extracts was obtained using "rotary evaporator" under cut down pressure. "Different concentrations were made viz., 10, 30, 50, 80 and 100 (mg/ ml) ethanolic as well as aqueous extracts were made by liquefy concrete extract in DMSO (10% Dimethyl sulfoxide)". Finally this prepared extract was preserved at temperature 4°C in a refrigerator.

Test microorganisms

Three species of bacterium comprises of "*Staphylococcus aureus* which is Gram positive bacterial strain and other two Gram negative bacteria namely *Escherichia coli* and *Pseudomonas aeruginosa"* were employed for antibacterial assay. The Bacterial were obtained from Division of Immunology and Microbiology Veterinary Sciences, SKUAST-K, Shuhama. Each two weeks of time all bacterium strains were conserve as well as manage for re-cultured them on nutrient Agar, at 2°C. Antibiotics like (Gentamycin discs) were received from SKUAST-K, Shuhama and worked as +ve control for antibacterium evaluation. Other side (DMSO) acted -ve control.

Antibacterial assessment

Antibiotic assessment for ethanolic as well as aqueous extracts was done, by the process "diffusion agar well procedure" as defined [48]. Uniformed 100 µl, inoculums of all test bacterial strains were inoculated on: (Agar), finally homogenized and poured into, sterile petri plates and form the uniform (4 mm) depth. Petri dishes were concede to get solidify in the laminar flow. Aseptic cork borers, of 4m were made to perform uniform as well as equidistant (wells) in every petridish. Concentration of 100 µl, (10 , 30 , 50, 80 and 100 mg ml^{-1} respectively) of plant corm extracts and prepared in (10% dimethylsulfoxide) were inserted into all different (wells). And middle the petridishes an antibiotic disc was placed i.e., (Gentamycin 10 µg/disc) which acted as (positive control), where as (10% DMSO) alone was used as (negative control) in an isolated petridish. Thereafter, these plates were incubated at 36°C for 16-25 hours. Finally zone of inhibition were recorded in each of the petridish, and potentional of (Antibacterial) property were figure out, by measuring the zone of inhibition area in (mm) millimeters by using (accepted measuring scale).

Statistical analyses

The results were evaluated statistically by applying: "analysis of variance (ANOVA)" with three factorial classifications: Bacterial Strains, Solvent Strains and Plant extract Concentration. The results presented in tables 2, were calculated.

Results

Antibacterial property of plant *Colchicum luteum* against different bacterial strains (mm) around the agar wells. Aqueous extract of *C. luetum* demonstrated best antibacterium action towards (*Pseudomonas aeruginosa* 13±1.84 mm) for inhibition zone area succeed by (*Staphylococcus aureus*, 16±0.25 mm) and (*E.coli*, 12±0.32 mm). Findings were correlated to a standard antibiotic (Gentamycin; drug), which exhibit area of inhibition counter to (15±0.35 mm *Pseudomonas aeruginosa*), (21±0.21 mm, *Staphylococcus aureus*) and (19±7.15 mm *Escherichia coli*) with concentration (100 mg ml^{-1}) corm extract (Table 1). 10% (DMSO) Dimethyl sulfoxide, acted as (-ve control) performed zero action towards each mentioned above bacterium strain.

Overall Ethanol extracts demonstrated best antibacterium action towards (*Pseudomonas aeruginosa* zone area of inhibition, 13±9.27 mm) succeed by (*Staphylococcus aureus* 16±0.25 mm) and (*Escherichia coli* 17±0.14 mm), findings were to a standard antibiotic (Gentamycin; drug), which exhibit area of inhibition (15±0.35 mm) counter to (*Pseudomonas aeruginosa* 25±0.23mm) against *Staphylococcus aureus* and (*E.coil* 25±0.17 mm) with concentration (100 mg ml^{-1}) corm extract respectively (Table 1). (DMSO) Dimethyl sulfoxide, acted as (-ve control) performed zero action towards each mentioned above bacterium strain.

"In the overall (ethanolic extracts) were much powerful towards each of the bacterium strain than (aqueous extracts) of *Colchicum luteum*" (Table 2).

Where as

S1, S2 and S3 = Bacterial Strains

C1, C2, C3, C4, and C5 = Different concentrations

CD (≤ 0.05)

Strain	= 0.1625
Solvent	= 0.13268
Concentration	= 0.2098
Strain x Solvent	= 0.2298
Strain x Concentration	= 0.36338
Solvent x Concentration	= 0.02967
Strain x Solvent x Concentration	= 0.511388

Discussion

Herbal plant species having antimicrobial properties are actually progressively described across worldwide. It is reported by WHO nearly "80% human population from developing countries full fill their initial strength very essential needs through local traditional antibiotic [49]. The two test methods using (agar- dilution procedures) were broadly exploit to determine the various antimicrobial actions of different samplings, consisting plants extract. As long as this idea, raw ethanolic corm extracts as well as consequent (fractions) of *C. luteum* were examined towards antibacterium actions work done by [50] and the present work was done on the herbal medicinal plant *Colchicum luetum* for its antibacterial properties.

The minimum inhibition concentration, MIC values in aqueous extracts performed best actions against (*Staphylococcus aureus*) and (*Bacillus subtilis*) reported by [51] further that the best antibacterial actions towards these bacterial strains. So, the Present research was undertaken as of developing (resistance to antibiotics) such as bacterial species as well as fungal species. Medicinal herbal extract as well as constituents of advanced concern as (ant infectious and antimicrobial) carriers . As an outcome, the antibacterial activities of herbal plant extract of *Colchicum luteum* was screened against the most common bacterial strains which include (*Staphylococcus aureus, Escherichia coli.* and *Pseudomonas aeruginosa*).

In present work Ethanolic extracts exhibit maximum antibacterium activities, against the (*Pseudomonas aeruginosa*) (Table 1-2) showed (zone inhibition) of (14±0.27 mm), these findings are related to an accepted (antibiotic drug) Gentamycin, which performed the (zone inhibition) of (15±0.35 mm) towards (*Pseudomonas aeruginosa*), at the concentration of 100 mg ml^{-1} plant corm extracts (Table 1-2). So, related work was also carried out by the other authors [50-53]. Therefore no work has been assessed before this research work on the same plant against the same selected bacterial stains, so there are enough references available for correlating the works accordance with other authors (Figures 1-6).

It was concluded that the *C. Luetum,* corm extract demonstrated a strong action towards the two strains of (gram +ve as well as gram –ve) also the findings were significantly positive and extracts of ethanol performed highest zone of inhibition activities towards these bacteria's related experiment were performed by Ahmad B, et al. [54] on medicinal plant (*Colchicum luetum*) in which he also receive better findings towards another bacterium "viz., *Bacillus subtilis, Shigella flexenari, Staphylococcus aureous, Escherichia coli, Klebsiella pneumonae,* and

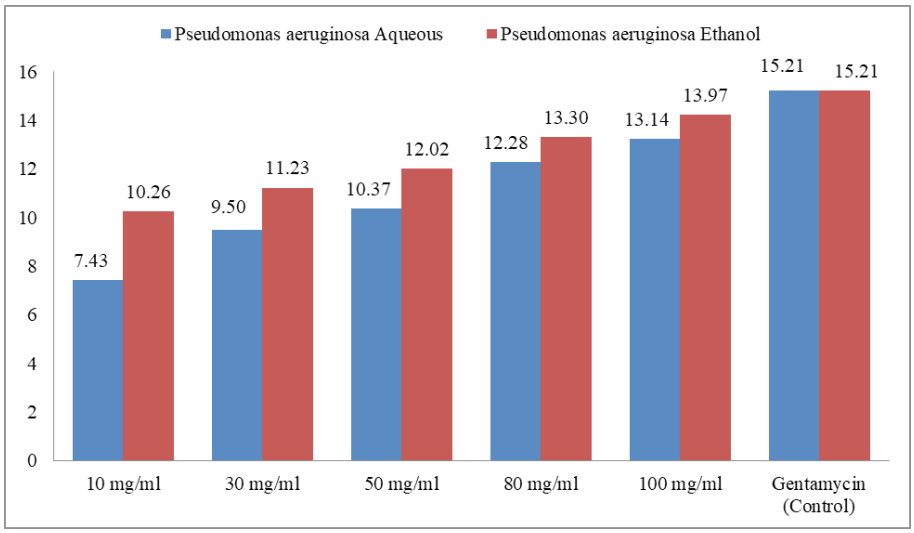

Figure 1: Antibacterial property of plant *Colchicum luteum* against *Pseudomonas aeruginosa* (mm) around the agar wells.

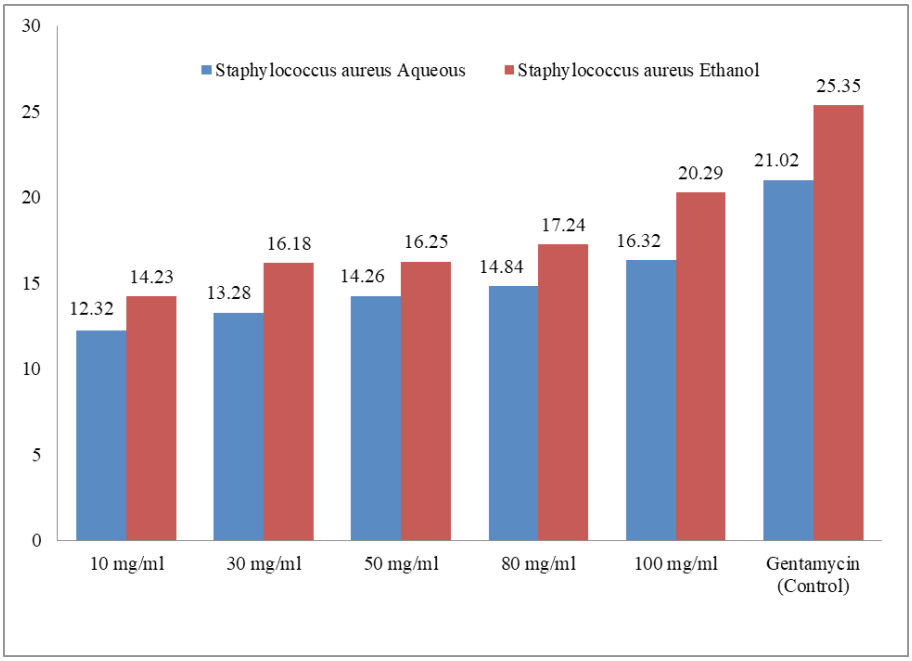

Figure 2: Antibacterial property of plant *Colchicum luteum* against *Staphylococcus aureus* (mm) around the agar wells.

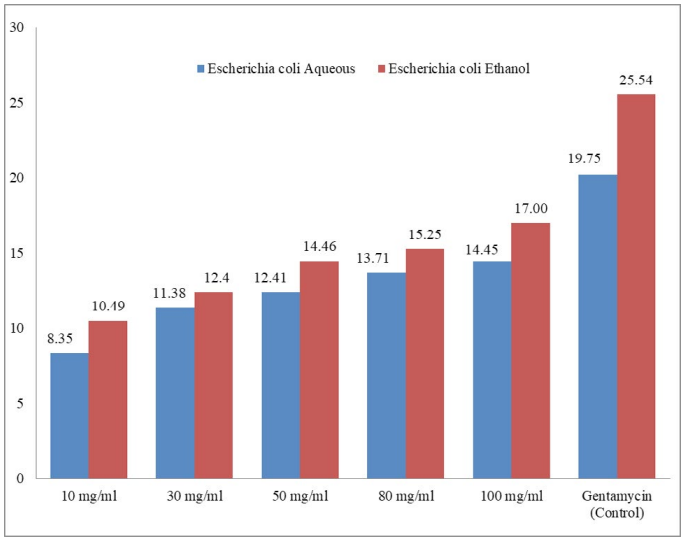

Figure 3: Antibacterial property of plant *Colchicum luteum* against *Escherichia coli* (mm) around the agar wells.

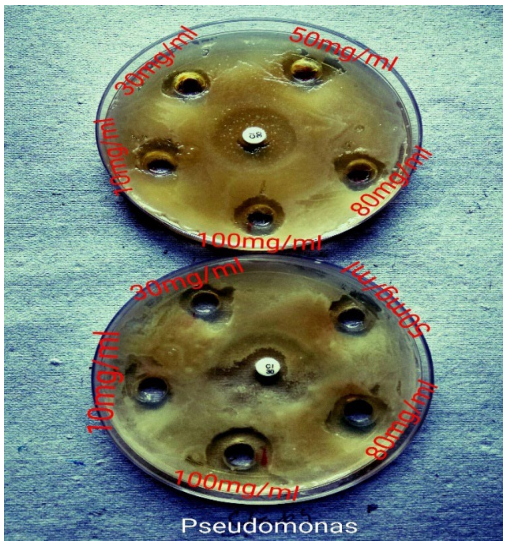

Figure 4: (Area inhibition, mm) neighboring (agar wells) against *Pseudomonas aeruginosa*.

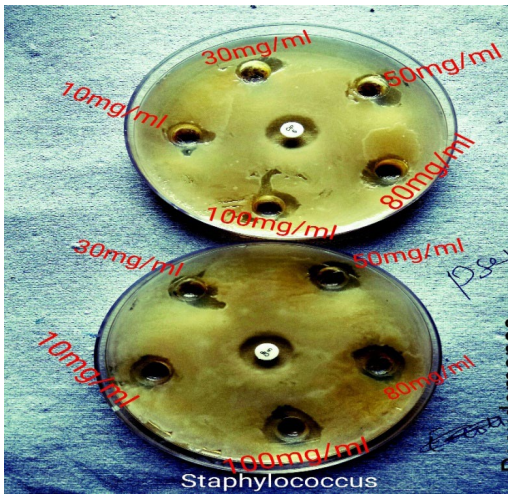

Figure 5: (Area inhibition, mm) neighboring (agar wells) against *Staphylococcus aureus*.

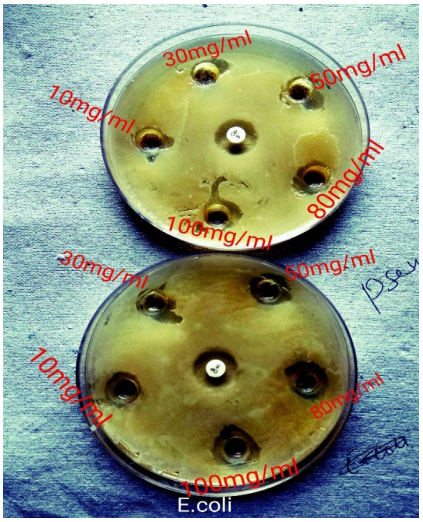

Figure 6: (Area inhibition, mm) neighboring (agar wells) against *Escherichia coli*.

Salmonella typhi". So, the experimental findings listed in (Figures 1-6), "this can be determined that this herbal species (*Colchicum luetum*) has magnificent antimicrobial (bacteria) activities towards mentioned above bacterium species, and results were significantly positive (Table 1 and 2).

Conclusion

The *Colchicum luteum* corm extract showed significantly positive actions against the mentioned (gram +ve as well as gram-ve bacterial strains. Ethanolic extracts demonstrated the best (zone of inhibition) towards bacterial strains. This concludes plant extract has lot of potentional against several bacterial diseases introduced by (*Pseudomonas aeruginosa*, *Staphylococcus aureus and Escherichia coli* in human beings as well in animals. *Colchicum luteum* extract is used as an organic antibacterial agent as this extract is organic and is Ecofriendly in nature and its action spretrum is specific. The findings indicate the potential use of extracts from the plant to develop new antibacterial products. It is corms and seeds could be a natural source for the pharmaceutical industry Therefore, pharmaceutical Industries can manufacture the drugs against these bacterial diseases.

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Author Contribution

RAR and HB drafted the experimental design. RAR, MAB, SAP,RQ,TAB performed drafted the manuscript and formatting and initial draft of manuscript text; RAR, NN, FAL, SB, SA, JIAB performed the statistical analysis; RAR, SQ also performed lab experimental analysis; helped in data collection, data analysis and. All authors read the manuscript before communication.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Table 1: Antibacterial property of plant *Colchicum luteum* against different bacterial strains (mm) around the agar well.

Bacterial strain	strain Solvent	Plant extract Concentration (mg ml^{-1})					Gentamycin (10 µg/disc) (Control)
		10	30	50	80	100	
Pseudomonas aeruginosa	Aqueous	7±0.44	9±0.27	10±0.26	12±0.31	13±1.40	15±0.35
	Ethanol	10±0.24	11±0.51	12±0.41	13±0.84	13±9.70	15±0.78
Staphylococcus aureus	Aqueous	12±2.32	13±0.33	14±0.21	14±0.81	16±0.25	21±0.21
	Ethanol	14±0.32	16±0.25	16±0.35	17±0.47	20±0.27	25±0.23
Escherichia coli	Aqueous	8±0.38	11±0.36	12±0.29	13±0.32	14±0.30	19±7.50
	Ethanol	10±0.12	12±0.36	14±0.34	15±0.27	17±0.14	25±0.17

Table 2: Antibacterial property of plant *Colchicum luteum* against different bacterial strains.

Strain solvent	Solvent / Concentration	C1	C2	C3	C4	C5	Mean	Factor mean of solvent
S1	Aqueous	07.43	9.50	12.28	10.37	13.14	10.56	**Aqueous**=13.32
	Ethanol	10.26	10.59	13.30	11.97	13.97	12.07	**Ethanol**= 14.29
	Sub mean	08.84	10.04	11.17	12.79	13.73	**11.32**	
S2	Aqueous	12.26	13.28	14.26	16.60	16.32	14.34	
	Ethanol	14.23	16.25	16.18	17.24	20.29	16.84	
	Sub mean	13.24	14.76	16.18	17.24	20.29	**15.59**	
S3	Aqueous	08.35	11.38	12.41	13.71	14.45	12.06	
	Ethanol	10.49	12.40	14.46	15.25	17.31	13.98	
	Sub mean	09.42	11.89	13.43	14.48	15.88	**13.02**	
	Mean	**10.50**	**12.23**	**13.27**	**14.56**	**15.97**		

Data availability statements

Data sharing does not apply to this article as no datasets were generated or analyzed during the current study.

Ethical approval

Not required

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