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Evaluation of *In vitro* Antimicrobial Activity of Extract of *Nigella sativa*, Moon flower and Yellow thistle

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Abstract

The increasing resistance of microbial pathogens to conventional antibiotics necessitates the exploration of alternative antimicrobial agents. This study evaluates the antimicrobial potential of a polyherbal methanol extract (PHME) formulated from *Nigella sativa*, *Datura stramonium*, and *Argemone mexicana*. The antimicrobial activity was assessed using the agar well diffusion method against gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacterial strains. Minimum inhibitory concentration (MIC) was determined using the broth dilution method. The results demonstrated a significant antimicrobial effect of PHME, showing a synergistic enhancement over individual plant extracts. PHME exhibited the highest inhibition zones and lowest MIC values among test samples, supporting its potential as a natural antimicrobial formulation.

Keywords: Antimicrobial activity, Polyherbal extract, Agar well diffusion, Minimum inhibitory concentration, *Nigella sativa*, *Datura stramonium*, *Argemone mexicana*

Introduction

The rise in antibiotic-resistant bacterial strains is a global health concern, driving the search for alternative antimicrobial agents derived from natural sources [1,2]. Medicinal plants have historically played a crucial role in combating infections due to their rich phytochemical compositions, including alkaloids, flavonoids, tannins, and terpenoids, which possess significant antimicrobial properties [3,4].

Several studies have highlighted the antimicrobial potential of *Nigella sativa*, *Moon flower*, and *Argemone mexicana*. *Nigella sativa* (black seed) contains thymoquinone, which exhibits broad-spectrum antimicrobial activity against various bacterial and fungal pathogens [5,6]. *Moon Flower*, rich in alkaloids such as scopolamine and atropine, has demonstrated antimicrobial and antifungal properties [7,8]. *Argemone mexicana* is known for its antimicrobial alkaloids, including berberine, which is effective against drug-resistant bacteria [9,10].

Polyherbal formulations offer the advantage of combining bioactive compounds from multiple plants, potentially enhancing antimicrobial efficacy through synergistic interactions [11]. This study evaluates the antimicrobial activity of a methanol-based polyherbal extract (PHME) against gram-positive and gram-negative bacterial strains using in vitro methods.

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Material and Methods

Selection, Procurement, and Authentication of Plant Materials

Medicinal plant specimens, including *Plumbago zeylanica* Linn, *Moon Flower*, and *Argemone mexicana* Linn, were collected from the campus of Mohanlal Sukhadia University, Udaipur, Rajasthan. To ensure botanical accuracy, the collected samples were prepared as herbarium specimens and authenticated by the Botanical Survey of India, Jodhpur, Rajasthan. The authenticated samples were subsequently deposited for future reference.

Preparation of Extract

The extraction process was carried out individually for the selected plants (*Nigella sativa* seeds, *Moon Flower* leaves, and *Argemone mexicana* aerial parts). The plant materials were shade-dried for two weeks to retain their phytochemical integrity, followed by pulverization into a coarse powder. The powder was then passed through a mesh size 20 sieve to maintain uniformity.

To remove fatty components, the coarse powder was subjected to defatting using petroleum ether (60–80°C) for 24 hours. After defatting, re-extraction was performed using methanol as a solvent in a Soxhlet apparatus under controlled conditions. The obtained extracts were filtered through Whatman filter paper, concentrated using a rotary vacuum evaporator, and subsequently stored in a desiccator to prevent moisture absorption. The percentage yield of each extract was calculated for further analysis.

Evaluation of Antimicrobial Activity:

In-vitro antimicrobial activity by Agar well diffusion method

Test microbial strains

All the test drugs were tested for their effect on gram positive strains (*Staphylococcus aureus*; MTCC737, *Bacillus subtilis*; MTCC1305) and gram-negative strains (*Escherichia coli*; MTCC1687 and *Pseudomonas aeruginosa*; MTCC1688) by using cup plate method.

Culture of test microbe

Forthecultivationofthebacterialstrains,nutrientbrothmedium(NBM) werepreparedusing8%nutrientbrothindoubledistilledwaterandagar-agar.Itwassubjectedto autoclavingat15 Ibs psi for25-30 mins. Agartest plates wereprepared bypouring15ml ofNBM intopetridishesunderasepticconditionandallowedto standatroomtemperatureforstabilization. Bacterialcellculturesweremaintainedinpeptonesalinesolutionbyregularsub-culturingandwas incubated at 37°C for 24hrs).

Preparation of agar plates and sampling of the test drugs

Agarplateswereinoculatedbystreakingofbacterialstrainsovertheentiresterileagarsurfacefor 2-3 times by rotating the agar plate at 60 degrees for uniform distribution of the inoculum. The plates were allowed to dry at room temperature and then 9 mm diameter wells were bored in the agar plates. Test drugs (PZME, DSME, AMME and PHME) in concentration of 50,100,150 and 200mg/ml respectively and standard drug Ofloxacin (10µg/ml) were prepared using dimethyl sulphoxide (DMSO) as diluting solvent. The standard and test drugs (100µl) wereadded in wells by using sterile micropipette. The plates were then incubated in a BOD incubator at37°Cfor24hrs.Thezoneofinhibitionwasmeasured byusingcalibrateddigitalVerniercalliper. The procedure was repeated in triplicate for each bacterial strain.

Determination of minimum inhibitory **concentration (MIC) by broth dilution method**

Serialdilutionsofextracts(50,25,12.5,6.25,3.12,1.56,0.78,0.39and0.19mg/ml)andstandarddrugofloxacin(10,5.0,2.5,1.25,0.62,0.31,0.15,0.07,0.03and0.01µg/ml)wereprepared.Each testtubecontaining100µlof105CFU/mlofteststrain(*Staphylococcusaureus,Bacillussubtilis, Escherichiacoli*and*Pseudomonasaeruginosa*)were inoculatedintubeswithequalvolumeofnutrient broth. The tubes were incubated aerobically at 37 °C for 24-48 hrs. 3 control tubes were maintained for each strain (media control, organism control and extract control). The lowest concentration of the extract that produced no visible growth (no turbidity) in the 24 hrs as compared to the control tubes were considered as MIC.The MIC was determined against all four selected microorganisms separately.

Results and Discussion

Screening of extracts by *in vitro* antimicrobial assays

Determination of zone of inhibition of individual and polyherbal extract by agar well diffusion technique.

The antimicrobial spectrum showed that all the test samples were found to be effective against both gram positive and gram-negative strains in a concentration dependent manner. The zone of inhibition of the test samples when compared at each concentration level for individual strains, it was found that YT exhibited antimicrobial efficacy with least zone of inhibition (ZI) against *S. aureus, S. bacillus, E. coli and P. aeruginosa* at 200 mg/ml. The sample PHME was found to be significantly more effective (Maximum ZI among all test samples) against S. aureus, S. bacillus, E. coli and *P. aureginosa*at maximum concentration level (200 mg/ml) which clearly showed the combinational effect of methanolic extracts. The results were showed antimicrobial efficacy in order viz. PHME > NS > MF > YT. Ofloxacin shows its maximum antimicrobial efficacy as standard drug as compared to PHME under consideration. The results showed the synergistic effect of PHME in comparison to individual methanolic fractions against both gram positive and negative strains. The antimicrobial activity of the selected plant extracts and its polyherbal composition were consistent with the ethnopharmacological relevance.

Table1:Antimicrobialefficacyofmethanolicextractagainstbacterial strains

Strains	Testdrug	Zone of inhibition (in mm), mean±SEM			
		50mg/ml	100mg/ml	150mg/ml	200mg/ml
S. aureus	NS	18.50±0.545 ****	20.50±0.514 ****	24.10±0.514 ****	25.00±0.824 ***
	MF	18.60±0.545 ****	21.20±0.465 ***	22.00±0.544 ****	23.30±0.874 ****
	YT	11.75±1.480 ****	13.00±1.154 ****	18.50±0.589 ****	19.32±0.200 ****
S. bacillus	NS	18.30±0.566 ****	21.75±0.520 ****	23.75±0.556 ****	24.00±0.521 ****
	MF	16.20±0.525 ****	18.40±0.573 ****	20.50±0.485 ****	21.75±0.580 ****
	YT	13.50±0.520 ****	15.25±0.560 ****	17.75±0.150 ****	18.75±0.100 ****
E. coli	NS	16.10±0.480 ****	18.20±0.532 ****	19.00±0.547 ****	20.55±0.510 ****
	MF	22.50±0.559	25.25±0.850	26.50±0.480	27.50±0.400
	YT	16.45±0.564 ****	17.10±0.570 ****	19.50±0.250 ****	20.50±0.560 ****
P. aureginosa	NS	16.30±0.558 ****	19.15±0.400 ****	22.50±0.529 ****	23.50±0.454 ****
	MF	16.40±0.514 ****	18.70±0.565 ****	20.50±0.540 ****	21.00±0.100 ****
	YT	11.50±0.458 ****	12.75±0.590 ****	15.50±0.555 ****	16.80±0.450 ****

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All values are represented as mean ± SEM, n = 3 for each group, Data were analysed by two-way ANOVA, for each bacterialstrain, followed by Dunnett's multiple comparisons test Multiple Comparisons Test,****p<0.0001, Asterisk (*) denotes significant difference as compared to test drug PHME.

Table 2:Antimicrobialactivityofstandarddrugagainstdifferentmicrobial strains

Ofloxacin (10µg/ml)	
S. aureus	31.00±0.816
S. bacillus	31.50±0.530
E. coli	32.50±0.577
P.aureginosa	33.75±0.500

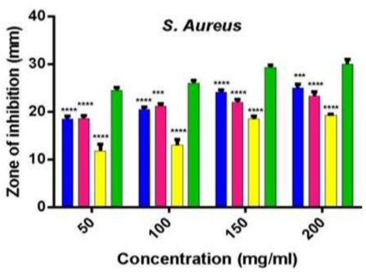

Figure1:Antimicrobialspectrumofdifferentplants extractandpolyherbalagainst*S.Aureus*

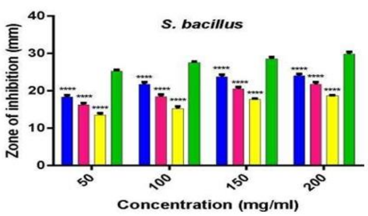

Figure2:Antimicrobialspectrumofdifferent plantsextractandpolyherbalagainst*S.bacillus*

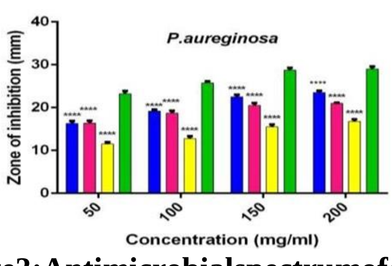

Figure3:Antimicrobialspectrumofdifferent plantsextractandpolyherbalagainst*P.aureginosa*

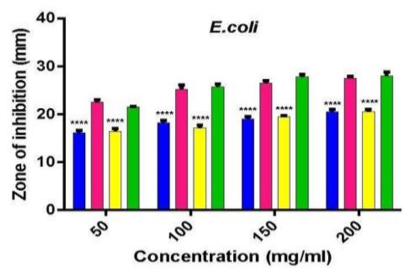

Figure4:Antimicrobialspectrumofdifferentplants extractandpolyherbalagainst*E.coli*

Antimicrobial efficacy of methanolic extract against bacterial strains at different concentration

All values are represented as mean ± SEM, in triplicate (n= 3). Data were analysed by one-way ANOVA, followed by Tukey-Kramer Multiple Comparisons Test.Data represented as significant difference as compared to PHME group and ****P < 0.0001.

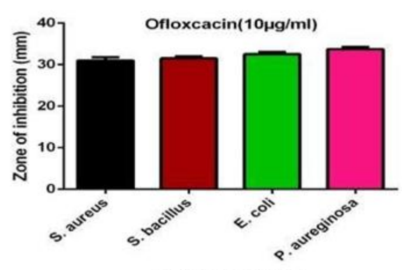

Figure5:Antimicrobialactivityofofloxacinagainstdifferentmicrobialstrainsat 10µg/ml

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DeterminationofMICofindividualandpolyherbalplantextractby Broth dilution method

MICs are used to evaluate the antimicrobial efficacy of test drugs by broth dilution method. Thismethodisusedtomeasuretheeffectofdecreasingconcentrationsoftestdrugintermsof inhibition of microbial growth. These evaluations can be used to determine appropriate concentrationsrequiredtoproducetheeffect.TheMICsofthetestdrugarequitelessthanthe concentrationfoundinthefinisheddosageform.The differentmethanolictestextractsshowed variableMICsagainstbothgramnegativeandgram-positivestrains.ThePHMEwasfoundto exhibitleastMICagainst*S.bacillus.E.coli*and*P.aureginosa*ascomparedtoNS,MFandYT. PHME showed the same MIC value as NS against *S. aureus*., The average MICs of allthemethanolicextractandcombinationswerefoundtobeinorderviz.PHME <NS <MF <YT(Fig.5.10)whichclearlyshowedaninverserelationwiththezoneofinhibition.Itcanbe concluded that PHME showed combinatorial effect with least MIC value. The phenolic and flavonoids contents of the selected herbs probably killed the microorganisms either through inhibitionofcellwallsynthesisordisruptioninpermeabilityofbacterialcellmembrane.These increased the probability of loss of membrane function and all key cellular constituents, resulting in mutation, cellular damage and finally cell death.

Table3:MinimumInhibitoryConcentrationofdifferentmethnolicextracts

Extracts	*S. aureus*	*S. bacillus*	*E. coli*	*Pseudomonas*	Average MIC
NS	3120 µg/ml	3120 µg/ml	1560 µg/ml	6250 µg/ml	3510 µg/ml
MF	1560 µg/ml	6250 µg/ml	780 µg/ml	6250 µg/ml	3690 µg/ml
YT	780 µg/ml	3120 µg/ml	1250 µg/ml	6250 µg/ml	5650 µg/ml
Ofloxacin	0.62µg/ml	0.31µg/ml	0.15µg/ml	1.25µg/ml	0.58 µg/ml

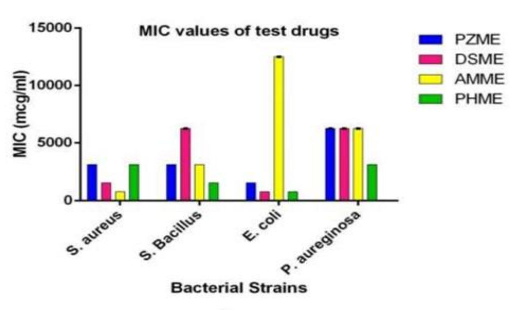

Figure6:Representedcomparativeminimuminhibitoryconcentration(MIC)valuesoftest drugs extracts against different bacterial strains

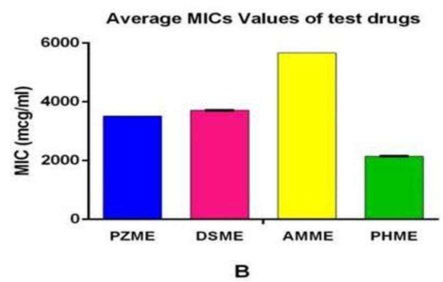

Figure7:Represented averageMICvaluesoftestdrugs

Conclusion

The results of this study indicate that PHME exhibits significant antimicrobial activity against both gram-positive and gram-negative bacterial strains. The synergistic effect observed in PHME suggests that the combination of *Nigella sativa*, *Datura stramonium*, and *Argemone mexicana* enhances antimicrobial efficacy beyond individual plant extracts. These findings support further in vivo studies and the potential development of PHME as a natural antimicrobial agent to combat antibiotic-resistant bacterial infections.

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