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The Analysis of Antibacterial and Antifungal Activity of Various Leaves Extracts of Natural Plants *Maesaindica* (Wall) against Bacterial and Fungal Strains Causing Human Infection

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Abstract: **The indigenous plants are used as traditional medicinal plants from decades, which are useful in treatment of numerous contagious diseases. Medicinal plant of our community could be a wonderful origin of drugs which can cure these disease effortlessly .Active compound which are present in plants are responsible for the biological activities of the natural plants during the metabolism plant species. So, this investigation was focused to analyze the antimicrobial activity from leaves extract of medicinal plants *Measaindica* wall (known a Nagaphadhera in Kumaon of the order *Myrsinacceae*. The antimicrobial activity against various pathogens was calculated by measuring minimum inhibitory count (MIC) and Zone of inhibition. Minimum bactericidal count (MBC) and minimum fungicidal count (MFC) value were determined as well. Every extracts showed the activity against various microorganism but Petroleum ether extract showed maximum zone of inhibition and (MIC) value. Ethanol & water showed minimum zone of inhibition and minimum inhibitory concentration (MIC) value. Therefore, Various solvent extract of the leaves of *Maesa Indica* have been found to possess antimicrobial activity and could be very effective agents for the isolation of better drugs to cure several antimicrobial diseases. The petroleum ether sample can be used to discover new drugs of very high potential.**

Index Terms: **Antimicrobial activity, Bacterial strain, fungal strains, i, Medicinal plants, MIC, MBC, MFC, and Zone of inhibition.**

I. INTRODUCTION

The Nature is the store house of natural product with the development of science and technology. New drug discoveries have shifted attention from synthetic model and compounds to natural products of plant origin (Kirti, et.al, 2017). Natural plant kingdom has great importance in life of human being with about 90% of the total world's population depends upon these bioactive drugs.

Herbal drugs have found wide spread use in country. These all natural plants are having great value in the medicinal science from the past several years (Nagendra K, et.al., 2012; Vankar P.S, et.al, 2008). Medicinal science has developed tremendously due to several research and discoveries of variety of natural and chemical based drugs. Herbal medicinal plant are full of bioactive material provide which are very important for the discovery and development of new cheaper and more effective drugs of original habitat. Hence, in the present research the antibacterial and antifungal activity of the leaves of plants M*asea indica* is calculated and found to have potential biological properties can be used for development of new medicinal drugs (Daha et al., 2019).

Maesa indica wall plant is commonly known as Nagapdhera in Kumaon in Malayalam and Jindali in Garhwali. It belongs to natural order *Myrsinaceae*. The berries of these plants are used as anthelmentic and the leaves are used as antidote to fish poison. The plant is very commonly in North East Himalayas. It generally occurs in East Bengal, Darjeeling, and Manipur. The leaves of plant *Mesaindica* wall was dried, powdered and subjected for extraction with petroleum ether, methanol, ethanol, petroleum ether and water as the solvent in a soxhlet extract for about 70 hrs at (40-60°C). After the extraction, all the samples were subjected for the examination of their antimicrobial activity (Rajput, et al., 2012). In view of this, that the present research work focused on the survey of antimicrobial

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activities of different leaves extract.

II. EXPERIMENTAL SECTIONS

A. Plant and Specimen Collections

The medicinal plant *Maes indica* was collected in curing various disease and its ethno medicinal values. The plant taken for the analysis should to free of any type of contaminations and physiologically well (Bhardwaj, V., et al.,2019).

B. Preparation of Plant Extracts

Powdered sample was blended with various solvents (Like Ethanol, Methanol, Petroleum Ether and Water) with respective to their increasing polarity continously for 12hrs in the soxhlet apparatus (Indrayan, A.K., et al., 2011). The temperature was not more than the boiling point of the each solvent. Then Extra liquid which is present during extraction process was separated by distillation process. The concentrated mass obtained was dried in incubator at 400°C.leftover distillate product was dried and dissolved in 50% DMSO (dimethyl sulfoxide) further which was preserved in cool storage at 40°C sterile glass tubes.

C. Microorganism Source Used for Test

The Various bacterial and fungal Pathogen (Latha et.al., 1998) taken for antimicrobial activity analysis was as in Table I.

Table I. Bacterial and Fungal Strains

S.No.	Bacterial Strains	Fungal Strains
1	*Bacillus Cereus*	*Aspergillusniger*
2	*S.aureus*	*Aspergillusflavus*
3	*Escherichia Coli*	*Rizopusstoionifer*
4.	*Pseudomonas aeruginosa*	*Fusariumoxisporim*

D. Media Composition & its Preservation

The antibacterial and antifungal activities was tested on solid (agar-agar) media in petriplates for bacterial assay nutrient agar (NA) (40gm/l) and fungus PDA (39gm/l) was used for developing surface colony growth. The suspension culture, for bacterial cell growth was performed by 2% (w/v) Lauria Broth and for fungus cell growth, 2.4% (w/v) PDB (Potato dextrose broth) was used. All media compositions were decontaminated by autoclaving at 125°Cfor 15-25 min. (Chopra, et al., 1980).

E. Agar Well Diffusion method

This method is widely used to examine the antimicrobial activity. The 8-10 hold cultured broth plates were smeared for bacteria and fungi respectively with Nutrient agar (NA) and potato Dextrose Agar (PDA) media. (Mann, et al., 1998) The Wells was digged in all the (10mm diameter and about 2 cm a part) plates with the help of sterile corn borer. Stock solution of various samples taken out was made by taking concentration of 1mg/ml in various plant extract that is methanol, ethanol, Petroleum ether, water. 80-100 ul of solution of various dilutions from various extract was poured with decontaminated syringe into the well and maintained at 37·c for 2-3hrs. Further all the plates were incubated at 37°C for 18-24hr for bacterial pathogen and 28°C for 46- 48 hr for fungal pathogen. The result was recorded nearby all the wells as measured of diameter of the zone of inhibition (mm). All the experimental process was performed in triplicate. The result was noted from the various directions & the average value was put down in the Table II.

F. MIC Values Analysis

The analysis of MIC values was carried out with help of the broth serial dilution method. (Carson et al., 1999) After incubation of plates the reading was noted for calculation. All the different extracts are taken in serial dilution with Luria broth

Table II. Zone of inhibition (mm) of all leave extracts of plant against microbial strains

Microorganism bacteria	**Ethanol**	**Methanol**	**Petroleum ether**	**Aqueous(water)**	**Standard(streptomycin)**
B.cereus	12.6	16.4	18.7	ND	22..6mm
S.aureus	11.8	15.5	16.4	13.6	19.3mm.
E.coli	11..9	12.5	17.5	14.2	21.7mm
P.aeruginosa	12.2	13.7	14.4	9.8	19.5mm
fungi	**Ethanol**	**Methanol**	**Petroleum ether**	**Aqueous(water)**	**Standard(griesoflvin)**
A.niger	12.5	12.1	15.3	12.6	16.33mm
A.flavus	10.9	14.3	13.5	11.3	15.21mm
R.stolonifer	11.6	12.3	14.6	ND	15.45mm
F.oxisporum	10.5	11.2	10.2	11.1	13.32mm

*ND: - not detected the growth of microorganism

for bacterium strains and PDB medium for the fungus strains. After the formation of media the test organism was poured in the serial dilution of the various extracts, further they all were incubated. After incubation the growth was measured. The extract no visible growth having minimum concentration is observed as the MIC Values.

G. Analysis of Antibacterial Activity

To evaluate the antibacterial activities of various compounds micro dilution method was used against various bacterial pathogens (Ahmad I., & Beg A.Z., 2001). The bacterial suspension was regulated with sterile saline to a concentration of $1*10^7$CFU/ml. various inoculums were made and were preserved at 4^{oc} for further use. To check the presence of the contamination all the dilution was subjected to culture on solid medium by this we can of check the contamination in the inoculums. Entire experiments was performed in triplicates stage with controls and repeated thrice.

H. Analysis of Antifungal Activity

To evaluate the antifungal activities of various compounds micro dilution method was used against various fungal pathogens (C. F. Carson, et, al., 1999).The fungi culture plate were properly clean from the top layer of the agar plates with sterile 0.85% saline containing 0.1% Tween 80 (v/v). The fungal suspension was directive with sterile saline to a concentration of $1*10^7$CFU/ml. various inoculums were made and were preserved at 4^{oc} for further use. (Hussian et al., 2011).

I. Determination of MBC

The MBC is the least concentration of an antimicrobial drug that is needed to finish bacterial pathogen. To calculate the MBC value serial sub-cultivation of 2µl in micro titer plates having 100ul of broth per well was used. After the formation of plates they were incubated for 72hours at 28^oC (Erlina et al., 2012). The plate which do not show visible growth and have the least concentration towards growth was considered as MBC value. To compare the results streptomycin was taken as standard drug for various bacteria strains as the positive control (Batra, et al., 2012). Entire experiments procedure was carried out in duplicate stage and was repeated thrice (Table III).

J. Determination of MFC

MFC considered as least concentration of an antifungal drug which is needed to finish any fungal pathogen. To calculate the MFC value serial sub-cultivation of 2µLmicrotiter plates having 100µl of broth per well was used. After the formation of plates they were incubated for 72 hours at $28^{0c.}$ (Ratnasooriya, et al., 2005). The plate which shows the no visible growth and has the least concentration towards the growth was considered as MFC value. To compare the results Greisofluvin (1-3000ug/ml) which is the standard drug used as positive controls. Entire experiments procedure was carried out in duplicate stage and was repeated thrice (Table IV).

III. Results and Discussion

Above mention research calculated the antimicrobial activity of various leaves isolates (methanol, ethanol, petroleum ether and water) of *Measaindica* were compared in opposite to microbial strains. The microbial activities were quantitatively evaluated on the basis diameter of zone of inhibition (mm) along with minimum inhibitory concentration (MIC) values evaluations. (Paulo C., et al., 2002). Evaluated values index

Table III. MIC(µg/ml) and MBC of the various leaves extracts of plant *Maesaindica* against bacterial pathogen

Microorganism bacteria	Ethanol		Methanol		Petroleum Ether		Aqueous (water)		Standard	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
B.cereus	22.4	44.8	20.4	42.7	30.4	54.4	18.8	53.6	36.7	57.9
S.aureus	18.4	51.8	33.5	63.6	22.4	42.8	16.4	50.7	23.4	60.6
E.coli	16.8	36.7	14.7	38.1	15.9	35.4	27.1	44.6	37.8	66.6
P.aeruginosa	10.2	49.6	16.3	42.2	19.4	56.8	22.2	52.3	24.3	72.8

Table IV. MIC (µg/ml) and MFC of all leaves extracts of plant Mesa indica against fungal pathogens

Microorganism Fungi	Ethanol		Methanol		Petroleum Ether		Aqueous (water)		Standard	
	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC	MIC	MFC
A. niger	18.2	36.5	22.6	39.7	19.7	54.4	18.8	53.6	36.7	57.9
A. flavus	20.3	33.3	15.3	52.1	24.3	42.8	16.4	50.7	23.4	60.6
R. stolonifer	16.5	31.7	12.2	23.2	15.8	35.4	27.1	44.6	37.8	66.6
F. oxisporum	15.5	35.4	13.9	28.8	16.3	56.8	22.2	52.3	24.3	72.8

were compared with the activity of the standard streptomycin (1mg/disc) for bacterial strains and Griseofluvin (1mg/disc) for fungal strains. (Ahmad I., et al., 2001). According to overall evaluation almost extracts can be explore as strong antimicrobial source of drug towards all microorganism strains which was taken.

CONCLUSION

The petroleum ether extract of *Measa indica* shows the significant antibacterial and antifungal activity against the various bacterial and fungal strains. These entire samples reveal maximum activity against in E. *coli* and B. *cereus* in bacterial strains & A. *niger* and R. *stolonifer* for fungal strain in Petroleum ether sample .The analysis also focused on the ethanol and water extract has average activity towards various bacterial and fungal strains, aqueous show the lowest activity against some strains. (Kamruzzaman, et. al., 2016). MIC study reveals that the samples are having variations in the values in various extracts.

According to this research work, the microbial strain those exhibits the highest value of MIC and lowest assay toward the MBC/MFC for the different extracts. (Nagendra K., et.al., 2012). The different microbial strain which exhibited the lowest value of MIC and highest value towards MBC/MFC for various extracts. In all result reveals that the extract are bacterostatic at least concentration but bactericidal at excessive concentration (Carson, 1999).The various ethanol, methanol, petroleum ether, and aqueous extract of the leaves plant .have bioactive compound which is available in this sample may be accountable for the antibacterial and antifungal behavior. The study indicates that different leave extracts of this plant may explore in future as strong and effective antimicrobial agents (Lakshmi V, et.al, 1987). These drugs can be explored as high potential antibacterial and antifungal agent at the cheaper cost in the future.

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