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Phytochemical, FTIR Analysis and Antibacterial Studies of Leaf Extract of *Rauvolfia Serpentina* Benth

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***Abstract:** This is the study of phytochemical, FTIR analysis and antibacterial studies of leaf extract of Rauvolfia serpentina. The species is commonly known as 'sarpagandha' or 'snakeroot' and belongs to apocynaceae family. Preliminary phytochemical screening of crude extract of leaves revealed the presence of chemical groups such as alkaloids, carbohydrates, saponins, glycosides, protein, phenolic compounds, phytosterol, flavonoids, terpenoids and tannins. The FTIR result revealed the presence of C-O, C–H rock bond, C–H bend or scissoring, the C=C stretch, C=O stretch, H–C=O stretch, C–H stretch, O–H stretch. It indicates the presence of secondary metabolites like alkaloids, flavonoids etc. The bacterial activities of Rauvolfia serpentina is tested against five main clinical pathogens; Escherichia coli(gram negative pathogen), Pseudomonas aeruginosa(gram negative pathogen), Klebsiella pneumonia (gram negative pathogen), Staphylococcus aureus(gram positive bacteria), Actinobactor sp(gram positive bacteria) and Enterococcus feacalis(gram positive bacteria). Result of antibacterial study also makes the plans as potential source for the extraction of several useful drugs of natural origin because synthetic drugs are known to have several side effects.*

I. INTRODUCTION

Rauvolfia serpentina Benth. (*R. serpentine*) is commonly known as 'sarpagandha' or snakeroot. It belongs to the apocynaceae family. It is an important ayurvedic shrub about 30 to 90 cm in height with white and pinkish flower. The plant is available in tropical regions of the Indian subcontinent and East Asia. *R. serpentine* contains many bioactive chemicals such as reserpine, ajmaline, yohimbine, serpentine, deserpidine, rescinnamine, etc. The root and leaf extracts of *R. serpentina* have a good medicinal value and are traditionally used as medicine in India. It is used for the treatment of various central nervous system disorders, high blood pressure, lack of sleeping, nervousness, hypertension, and various mental disorders. It resists prostate cancer, too. It is also used for snake bite. It is the most plant used in traditional Chinese medicine.

Reserpine is widely known bioactive compound is extracted from *Rauvolfia serpentina.* One of the aims is to evaluate the antimicrobial activity of *Rauvolfia serpentina* against *Enterococcus faecalis, Escherachia coli, Actinobacter sp., Klebsiella sp, NSSA* and *Pseudomonas sp.* Medicinal plants are traditionally used globally as remedy for the treatment of various diseases which include asthma, gastrointestinal symptoms, skin disorders, respiratory and urinary problems, and hepatic and cardiovascular disease. The plants have the ability to synthesize biologically active compounds which helps the plants to survive against adverse conditions such as temperature, water stress, pressure, insect and pest attack and microbial attack. The composition of biologically active compounds of medicinal plants varies broadly depending on the plant species, type of soil and their association with microbes. The study of the mechanism of antimicrobial action of medicinal plant extracts is the first step in the optimal utilization of these extracts as natural antimicrobial agents to extend the shelf-life and keep the food quality. The antimicrobial activity of plant extracts and phytochemicals was estimated with antibiotic susceptible and resistant microorganisms. In addition, the feasible synergistic effects when associated with antibiotics had been studied.

The study conducted by Aniel and Lagudu suggested that methanol extract of *Rauvolfia serpentina* roots would be helpful in treating diseases caused by human pathogenic bacteria and fungi. In particular, it can be recommended that the *R. serpentina* roots to be used for the control of infectious disease caused by multidrug resistant *Staphylococcus aureus* (Aniel K and Mutyala laguda., 2016). *R.serpentina* cultivated in uttarakhand has good reserpine content and also exhibited moderate to strong antibacterial activity against tested human pathogenic bacteria (Negi et al., 2014). The work conducted by Shakti Mehrotra and his group reported that improved perceptive of biosynthetic genes and biochemical pathways will definitely enhance the utility of *R. serpentina* hairy root cultures for medicinally important TIA production (Shakthi mehrotra et al., 2014). K. Lingaraju and his team conducted synthesis of green approached CuO nanoparticles using aqueous leaf extract of *Rauvolfia serpentina* as a fuel by solution combustion method has been successfully carried out (K. Lingaraju et al., 2015). Reserpine content in in vitro grown callus was enhanced by low dose of AlCl3 elicitor in *Rauvolfia serpentina*.

The increased antioxidant enzyme activities in response to applied elicitor make it evident that an oxidative stress is induced in cultivated tissues. Hence, AlCl3 elicitation represents a positive strategy in causing stress to cells/tissues, and may be utilized for enhanced secondary metabolite synthesis in plant cultures (Nadia Zafar et al., 2017). P. Bhagath and his group reported that optimized *Rauvolfia serpentina* hairy root culture system is a promising technology for further higher production of ajmalicine as compared to other established technology (P. Bhagath et al., 2019).

II. MATERIALS AND METHODS

A. Chemicals, Reagents and plant Source

The leaves of *Rauvolfia serpentina* were collected from Thanjavur. The plants were taxonomically identified and authenticated by Dr. M. Kamaraj.

Zinc oxide solution of 99.5% purity and methylene blue was purchased from Trichy. Molecular grade water (Millipore. Milli Q) was used throughout the experimental studies. All the glassware used in the present study was carefully acid washed and rinsed with Mili Q water.

1) Preliminary Phytochemical Analysis

a) *Preparation Of Plant Extract:* The aerial parts were washed under running tap water to remove the dust particles and were shade dried at room temperature. It was then powdered using an electrical blender and stored in sterile bottles, sealed tightly to prevent microbial attack, until use. 7g of the powder was transferred into a clean conical flask and dissolved in 70ml of ethanol. The powdered leaf samples were subjected to cold extract with ethanol. It was kept in orbital shaker for 24 hours. The solution acquired was kept open for evaporation. Once the filtrate has completely evaporated, it was sealed tightly, labeled and kept at 4 degree Celsius. The extracts thus obtained were used for phytochemical screening.

b) *Phytochemical Screening Of Plant Extract:* The extract was dissolved in glacial acetic acid and few drops of ferric chloride and concentrated sulphuric acid are added. A reddish brown colored solution at the junction of two layers and a bluish green color in the upper layer indicates the presence of glycosides.

c) *Test for Alkaloids (Wagner's test):* 1 ml of each extract was taken and added 3-5 drops of Wagner's reagent and observed for the formation of reddish brown precipitate or coloration.

d) *Test For Carbohydrates (Molisch's Test):* 1 ml of each extract was taken and added 3-5 drops of Molisch's reagent, along with this added 1 ml of concentrated H2SO4 down the side of the test tube. Then allowed the mixture to stand for 3 minutes. Observed for the formation of red or dull violet color at the interface of the two layers.

e) *Test For Cardiac Glycosides (Keller Kelliani's Test):* 1 ml of each extract was taken and treated it with 1 ml of glacial acetic acid and 2-3 drops of 5% ferric chloride solution. To this mixture added of 0.5 ml of conc. H2SO4. Observed for the formation of brown ring at the interface shows the presence of deoxysugar characteristic of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish ring may form.

f) *Test for flavonoids (Alkaline reagent Test):* 1 ml of each extract was taken and treated it with 3-5 drops of 20% NaOH solution. Observed for the formation of intense yellow color, which becomes colorless on addition of 0.5 ml dilute HCl indicates the presence of flavonoids.

g) *Test For Phenols (Ferric Chloride Test):* 1 ml of each extract was taken and added 5-6 drops of 5% aqueous ferric chloride solution and observed for the formation of deep blue or black color.

h) *Test For Amino Acid And Proteins (1% Ninhydrin Solution In Acetone):* 1 ml of each extract was taken and added 2-5 drops of Ninhydrin solution and kept it in a boiling water bath for 1-2 min and observed for the formation of purple.

i) *Test For Saponins (Foam Test):* 1 ml of each extract was taken and mixed with 5ml of Distilled water and shaken vigorously. Observed for the formation of persistent foam for 10-15 min. that confirms the presence of saponins.

j) *Test For Tannins (Braymer's Test):* 1 ml of each extract was taken and mixed it with 1 ml of 10% alcoholic ferric chloride solution and observed for formation of blue or greenish color.

k) *Test For Terpenoids (Salkowski Test):* 1 ml of each extract was taken and added 0.5 ml of chloroform along with 3-5 drops of conc. H2SO4. Observed for formation of the reddish brown precipitate.

l) *Test for Phytosterols:* 1 ml of each extract was added to 1 ml of concentrated sulphuric and allowed to stand for 5 minutes. Formation of golden yellow color in the lower layer, after shaking, indicates the presence of phytosterols.

2) *Anti-Bacterial Activity:* Plant products are gaining importance as bactericides and fungicides based on their systemic activity and low photo-toxicity. A large number of plants are known for their antibacterial and antifungal activity, hence, an attempt was made to study the antibacterial and pharmacological properties of different extracts of *Rauvolfia serpentina*. Antibacterial activity of different extracts of *Rauvolfia serpentina* was assessed against *Enterococcus faecalis, Escherachia coli, Actinobacter sp., Klebsiella sp., NSSA* and *Pseudomonas sp*. The following materials are used Nutrient agar medium, Sterilized Petri dishes, Pipettes of 0.1 – 0.2ml, Cultures, nutrient broth and Sterilized test tubes containing solutions of extracts of known concentration.

a) *Preparation of Media:* Nutrient agar medium was prepared by dissolving bacteriological peptone (5g), Beef extract (3g), sodium chloride (3g) and agar (20g) in distill water to produce one liter of medium. Then, it was decontaminated for 30 minutes at 15 lbs pressure. The pH of the solution was adjusted to 66-70 by using 40% NaOH and HCl.

b) *Preparation of Sub-Cultures:* The organisms used in the present study for testing the antibacterial activity of the extracts were obtained from the laboratory stock. On the day of testing, the organisms were sub-cultured into sterile nutrient broth. After incubating the same for 3 hours, the growth thus obtained was used as inoculums for the test.

c) *Sterilization of Media:* The media used in the current study, nutrient agar and nutrient broth were sterilized in a conical flask of appropriate capacity by autoclaving at 15 lbs pressure for 20 minutes. The cork borer, petridishes, test tubes and pipettes were sterilized by employing hot air oven at 160ºC for 1 hour.

d) *Preparation of test Solutions:* Solutions of all the extracts were arranged in distilled DMF and tested at the concentration 1mg/ml.

e) *Method of testing – Disc Diffusion Method:* With the help of a sterile borer, five cups of each having 8mm diameter were punched and scooped out of the test agar (the cups were numbered for the particular concentration of extracts and standards). Using disinfected pipettes 0.1ml, of the prepared standard and the sample solution were feel into bored cups. The dishes were left standing for 1-4 hour at room temperature as a period of pre-incubation of the different solutions. These were then incubated for 24 hours at 37ºC. The zone of inhibition established, if any, was then precisely measured and recorded.

3) *FTIR (Fourier-transform Infrared Spectroscopy):* The extracts ad been tested under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts have been centrifuged at 3000 rpm for 10 min and filtered through Whatman filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the DMSO(Dimethyl sulfoxide). The extracts were examined in the wavelength within 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were identified. FTIR analysis was made using Perkin Elmer Spectrophotometer system, which was used to identify the characteristic peaks in ranging from 400-4000 cm-1 and their functional groups. The peak values of the UV and FTIR have been recorded. Each analysis was repeated twice for the confirmation of spectrum.

III. RESULT AND DISCUSSION

A. *Preliminary Phytochemical Analysis Of Crude Extracts Of Rauvolfia Serpentina*

Phytochemicals	RSL		
	A	M	C
Alkaloids	+	_	+
Carbohydrates	+	+	+
Saponins	+	_	_
Glycosides	+	_	_
Protein	+	+	_
Phenolic Compounds	+	_	_
Phytosterol	+	+	+
Flavanoids	+	+	+
Terpenoids	+	+	+
Tannins	+	_	_

"+" = presence of compound
"-" = absence of compound.RSL = *Rauvolfia serpentina* leaf
A = Acetone
M = Methanol
C = Chloroform

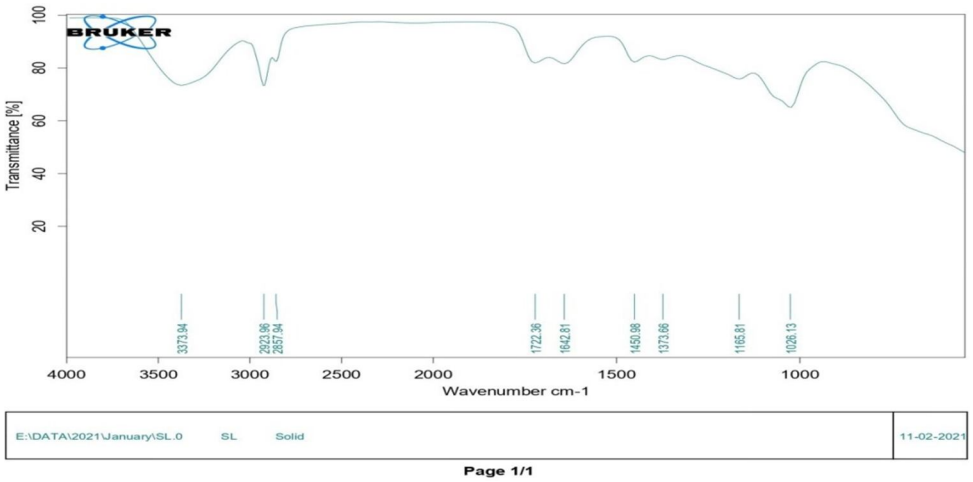
Preliminary phytochemical screening of the crude extracts of leaves of *Rauvolfia serpentina* revealed the presence of different kind of chemical groups.

1) *Alkaloids:* It was observed that alkaloids were present in all the extracts except methanolic extract. Alkaloids are recognized for use for the remedy of tumors, nocturnal leg cramps due to vascular spasms and diarrhea. These compounds own anti-microbial activity and sedative effects. Many alkaloids are anesthetics and have calming effects on or hypertensive patients without inducing sleep. Psychotic Alkaloids also can be used to deal with psychiatric and palpitation.
2) *Carbohydrates:* Carbohydrates were present all extracts.
3) *Saponins:* Saponins were present in acetonic extract. Saponins arefound in many plant-derived foods and medicinal plants and are heterogeneous group of natural products. Plant extracted saponins extracted has been reported to have significant biological and pharmacological activities such as anti-inflammatory, anti- hepatotonic, wound healing, veinotonic, expectorant, spasmolytic, hypoglycemic, antimicrobial and antiviral.
4) *Glycosides:* Glycosides were found to be present in the acetonic extract. Glycosides are usually small organic molecules in which asugar is bound to a non-carbohydrate moiety. Glycosides can suppress and soothe irritant dry coughs. When taken in small doses, they have a sedative and relaxant effect on the heart and muscles. They are also reported to be significantly diuretic.
5) *Proteins:* Proteins were present in all the extracts except chloroform extract.
6) *Phytosterols:* Phytosterols were found in all extract. Steroids present in plants is known as phytosteroids. Steroids are organic compounds with four cyclohexane rings. These steroidal compounds have been used to lessen stress, lessen cholesterol levels, and spark off immune system, increase memory and learning and to deal with tumor cells in cancer cases.
7) *Phenols:* Phenols were found to be present in acetonic extract. Phenols are one of the most common and widespread group of compounds as plant secondary metabolites. Phenols are antiseptic and decrease inflammation when received internally. Phenols are reported to have a high affinity to chelate metals and scavenge the free radicals in cells, which make them excellent anti-oxidant agents.
8) *Flavonoids:* Flavonoids were observed to be present in all the extracts. The flavonoids are a large group of naturally occurring polyphenolic compounds found in fruits, vegetables, grains, bark, roots, stems, flowers, tea, and wine'. Polyphenolic compounds present in plants are found have effective antioxidant properties, which protect cells against damage caused by the free radicals. It has been reported that these compounds deactivate the substances that promote the growth of tumors.
9) *Terpenoids:* Terpenoids were found to be present in all the extracts. Terpenoids are small molecular and are probably the most widespread group of natural products, synthesized by plants. Terpenoids has been reported to show significant pharmacological activities, such as antiviral, antibacterial, anti-inflammatory, antimalarial, inhibition of cholesterol synthesis and anti-cancer activities.
10) *Tannins:* Tannins were observed to be present in acetonic extract. Tannins are members of polyphenol chemical family. Tannins are known to be produced by all the plants, to a greater or lesser extent. They draw the tissues together and thus improving their resistance to infection. Tannin compounds present in WS has been reported to inhibit the growth of many fungi, yeast, bacteria and viruses. Tannins are attributed with analgesic and anti-inflammatoryactivities. Apart from this, tannins can heal the wounds and infected mucous membrane with the power of astringency.

B. FTIR

The fundamental measurement obtained in infrared spectroscopy is aninfrared spectrum, which is plotted as an infrared intensity versus wavelength (or frequency) of light graph. Transmittance is given in Y-axis and wavenumber is given in X-axis. Wavenumber is ranged from 1000 to 4000. In this fingerprint region start from 1000 to 1500.The spectra analyses shows some grooves in some regions such as 1165.81, 1373.66, 1450.98, 1642.81, 1722.36, 2857.94, 2923.96 and 3373.94. These wavenumbers are belongs to specific functional groups. Groove in 1165.81 cm-1 indicate the presence of strong band of the C-O stretch. Groove in 1373.66 cm-1 indicate the presence of strong band of the C–H rock bond. Groove in 1450.98 cm-1 indicate the presence of strong band of the C–H bend or scissoring. . Groove in 1642.81 cm-1 indicate the presence of strong band of the C=C stretch. Groove in 1722.36 cm-1 indicate the presence of strongband of the C=O stretch. Groove in 2857.94 cm-1 indicate the presence of strong band of the H–C=O stretch. Groove in 2923.96 cm-1 indicate the presence of strong band of the C–H stretch. Groove in 3373.94 cm-1 indicate the presence of strong band of the O–H stretch, hydrogen bonded. Fourier Transform Infrared Spectroscopy

The peaks at higher wave number region 2922, 2921, and 3294.78 cm-1 were associated respectively with stretching vibrations of C–H and OH groups of phenol/alcohol, which reveal the presence of the compounds like alkaloids, flavonoids etc.



C. Antibacterial Activity of PLANT Extract

CONCENTRATION	BACTERIA STRAIN											
	Escherichiacoli		*Pseudomonas*		*Klebsiella*		*methicillin-susceptible Staphylococ cus aureus*		*Actinobacteria*		*Enterococcus*	
20µl	9mM	8mm	7mm	8mm	5mM	6mm	8mm	8mm	10mm	8mm	11mm	12mm
	8mM		9mm		7mM		9mm		6mm		13mm	
	7mM		8mm		6mM		7mm		8mm		12mm	
40µl	10mM	11mm	12mm	13mm	9mM	8mm	11mm	12mm	11mm	12mm	13mm	14mm
	12mM		14mm		7mM		13mm		13mm		15mm	
	11mM		13mm		8mM		12mm		12mm		14mm	
60µl	13mm	13mm	16mm	15mm	10mm	11mm	13mm	13mm	13mm	14mm	14mm	15mm
	13mM		14mm		12mM		13mm		15mm		16mm	
	13mm		15mm		11mm		13mm		14mm		15mm	
80µl	14mM	15mm	19mm	18mm	11mM	12mm	15mm	16mm	16mm	16mm	18mm	17mm
	16mm		17mm		13mm		16mm		17mm		16mm	
	15mM		18mm		12mM		17mm		15mm		17mm	
CONTROL	22mm	20mm	23mm	22mm	0mm	0mm	12mm	13mm	16mm	16mm	27mm	28mm
	20mm		21mm		0mM		14mm		16mm		29mm	
	21mm		22mm		0mm		13mm		16mm		28mm	

The bacterial activities of *Rauvolfia serpentina* is tested against five main clinical pathogens; *Escherichia coli*(gram negative pathogen), *Pseudomonas aeruginosa*(gram negative pathogen), *Klebsiella pneumonia* (gram negative pathogen), *Staphylococcus aureus*(gram positive bacteria), *Actinobactor sp*(gram positive bacteria) and *Enterococcus feacalis*(gram positive bacteria). Figure illustrates zones of inhibition of these pathogens against standard drugs and ethanol extract of plant leaves dissolved in Dimethyl sulphonic oxide (DMSO) at concentrations 20 µl, 40 µl, 60 µl and 80µl. The mean values of zone of inhibition (mm) of three replicates are presented in table comparisons between standard antibiotics and methanol extract of *Rauvolfia serpentina* dissolved in DMSO compared to standard drugs.

In *Escherichia coli*, zone of inhibition of standard drugs ranged from 20mm to 22mm while that of plants extract was 7mm to 17mm. In *Pseudomonas aeruginosa*, zone of inhibition of standard drugs ranged from 21mm to 23mm while that of plants extract was 7mm to 19mm. In *Klebsiella pneumonia*, zone of inhibition of standard drugs ranged from 0mm to 0mm while that of plants extract was 5mm to 13mm. In methicillin susceptible *Staphylococcus aureus*, zone of inhibition of standard drugs ranged from 12mm to 14mm while that of plants extract was 7mm to 17mm. In *Actinobactor sp*, zone of inhibition of standard drugs ranged from 16mm to 16mm while that of plants extract was 6mm to 17mm. In *Enterococcus feacalis*, zone of inhibition of standard drugs ranged from 27mm to 29mm while that of plants extract was 11mm to 18mm.

Except *Enterococcus fecalis* showed minimum inhibitory concentration (MIC) at 20µl for ethanol extract of plant leaves dissolved in DMSO. Furthermore, as the concentration of plant extract increased so did the zone of inhibition. It is evident from the recorded images and statistical data that zone of inhibition of *Rauvolfia serpentina* mediated plant extract dissolved in DMSO against *Enterococcus feacalis* as compared to *Klebsiella pneumonia*. The mild inhibitory effect of *Rauvolfia serpentina* mediated plant extract dissolved in DMSO on *Klebsiella pneumonia* when compared to *Enterococcus feacalis* can be attributed to the differences in membrane structures of Gram-positive and Gram-negative bacteria.

E. coli can be attributed to the differences in membrane structures of Gram-positive and Gram-negative bacteria. The most distinctive feature of Gram-positive bacterium is the thickness of cell wall dueto the presence of peptidoglycan layer. It has also been reported that *Rauvolfia serpentina* mediated plant extract dissolved in DMSO may damage bacterial cell membrane resulting lysis of intracellular contents and ultimately proved to be lethal for the bacterial cell. Lower efficacy of *Rauvolfia serpentina* mediated plant extractdissolved in DMSO against *Enterococcus feacalis* compared to the Gram-negative species might be due to the resistance of cell wall in Gram-positive species. These results suggest that the use of *Rauvolfia serpentina* mediated plant extract dissolved in DMSO can be more efficient against gram-positive pathogens like *Enterococcus faecalis.*

IV. CONCLUSION

The result formulated from present study reveals that acetonic, methanolic and chloroformic extract of leaves of *Rauwolfia serpentina* contain several bioactive constituents including flavonoids, tannins, phenols, saponins, steroids, terpenoids, alkaloids, glycosides and reducing sugars which could be rendition for their diverse medicinal properties like anti-inflammatory, anti-spasmodic, antimicrobial, neuroprotective and diuretic effects. The FTIR result revealed the presence of C-O, C–H rock bond, C–H bend or scissoring, the C=C stretch, C=O stretch, H–C=O stretch, C–H stretch, O–H stretch. It indicates the presence of secondary metabolites like alkaloids, flavonoids etc. Antibacterial and related works also gave good results. This makes the plants as potential source for the extraction of several useful drugs of natural origin because synthetic drugs are known to have several side effects. However more clinical trials should be conducted to support their therapeutic use.

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A

B

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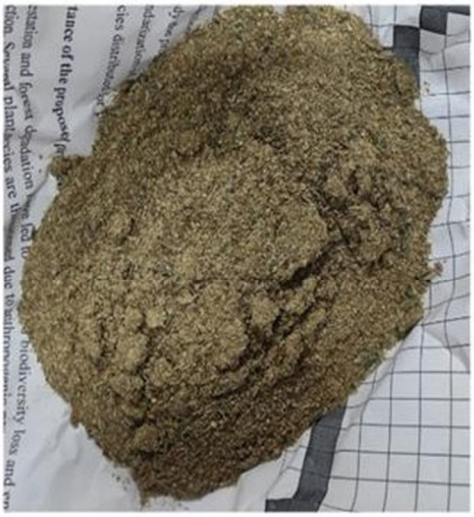
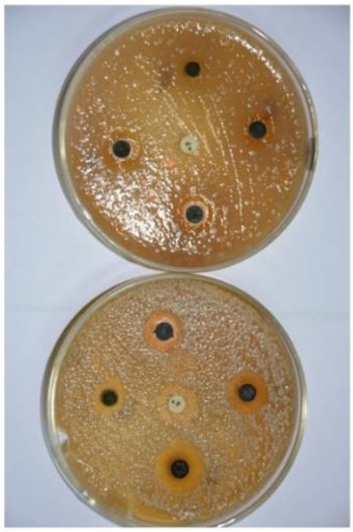
D



E

A	-	Plant leaves of *Rauvolfia serpentina* under shadow.
B	-	Pant extract in liquid form.
C&D	-	Soxhlet extractor.
E	-	Plant powder after extraction.

ANTIBACTERIAL STUDY



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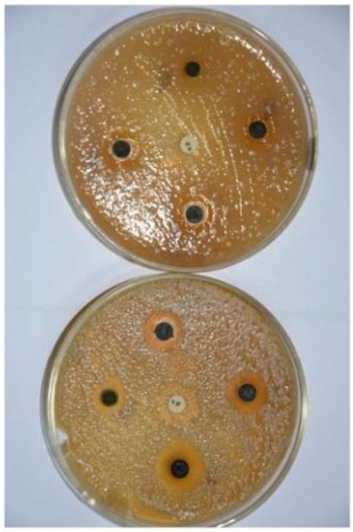

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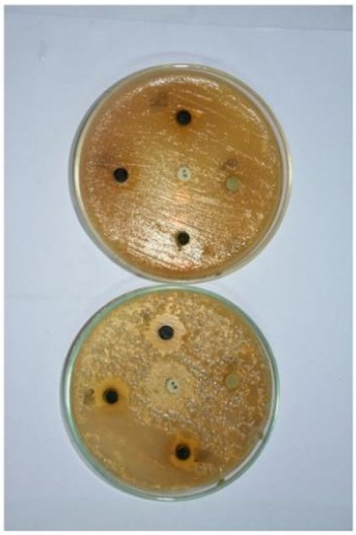

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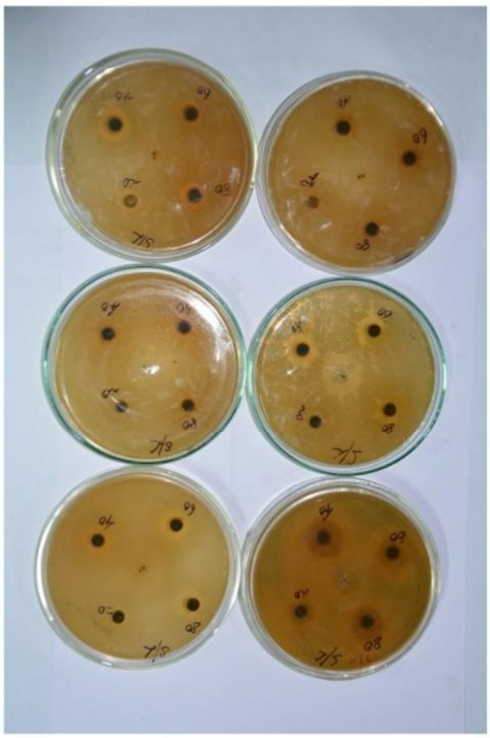

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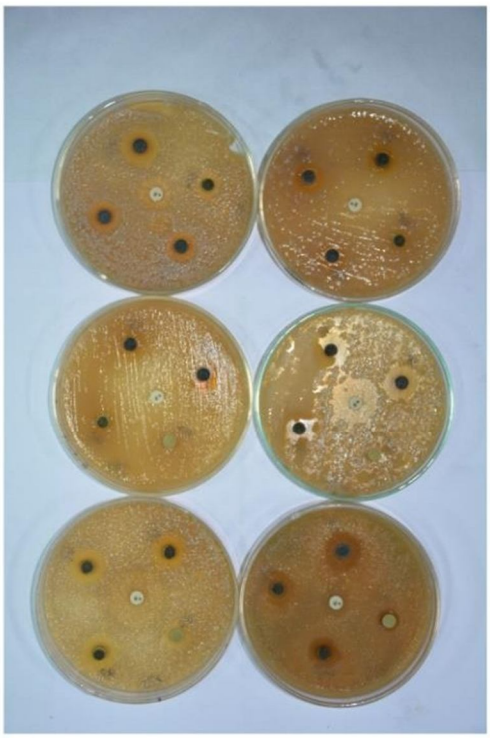

E

A- Enterococcus faecalis, Escherachia coli.
B- Actinobacter sp., Klebsiella sp.
C- NSSA and Pseudomonas sp.

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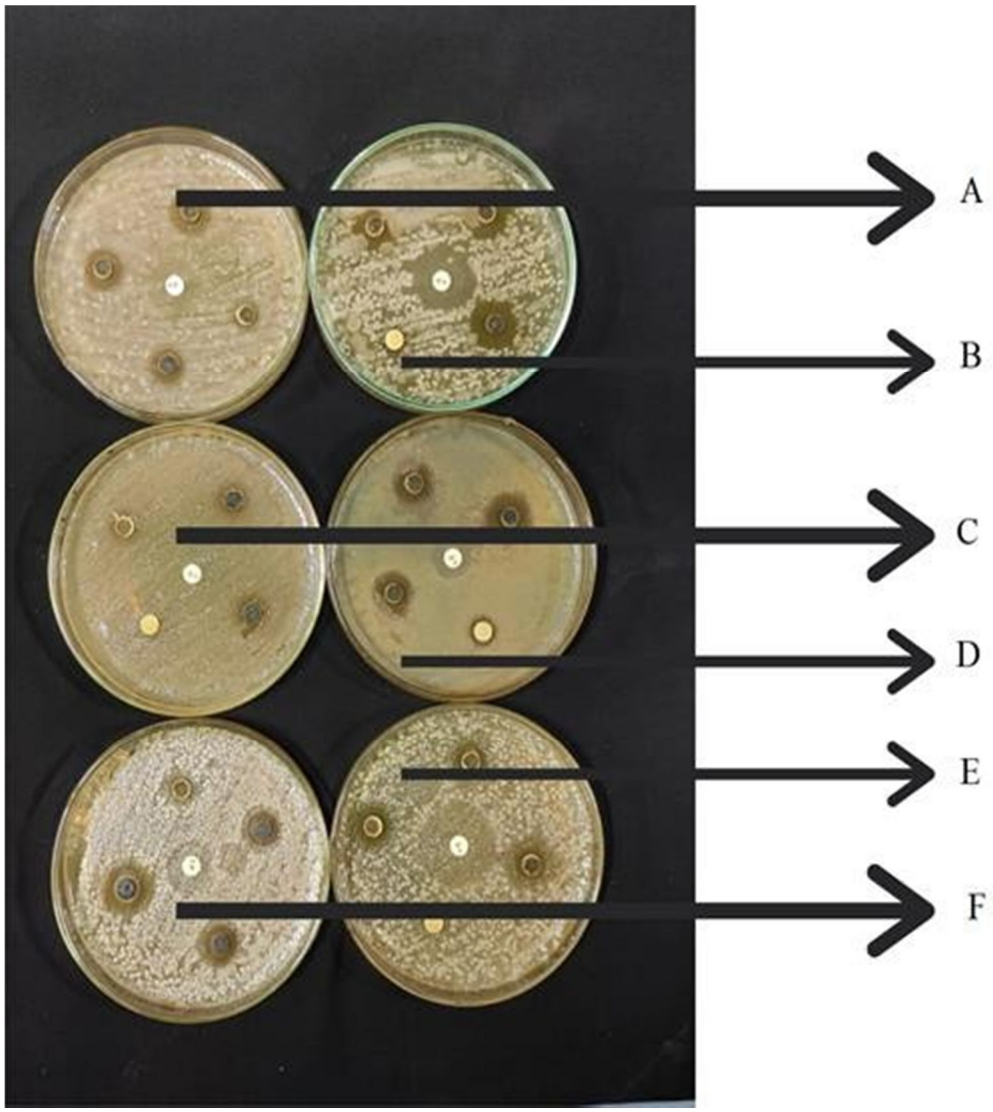
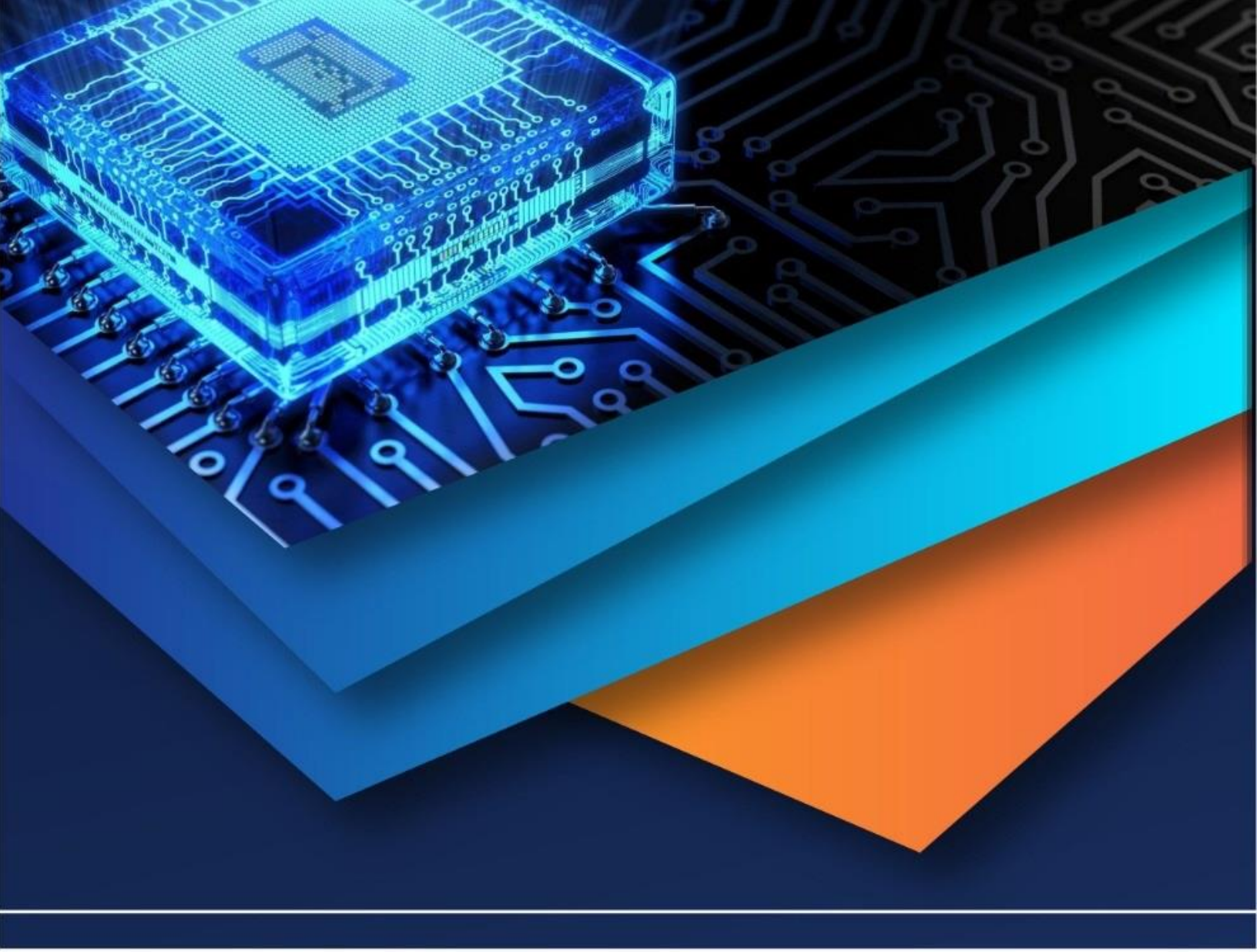


A - *Escherichia coli.*
B - *Pseudomonas aeruginos.*
C - *Klebsiella pneumonia.*
D - *Staphylococcus aureus.*
E - *Actinobactor sp.*
F - *Enterococcus feacalis.*



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IMPACT FACTOR: 7.129

IMPACT FACTOR: 7.429

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