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ANTIMICROBIAL ACTIVITY OF *NYCTANTHES ARBOR-TRISTIS* AGAINST *STAPHYLOCOCCUS AUREUS, STREPTOCOCCUS PYOGENS, PSEUDOMONAS AERUGINOSA* AND *SALMONELLA TYPHI*

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Keywords:

Nyctanthes arbor-trisitis, Geographical locations, Medicinal plants, Phytochemical properties, Extract preparations, Antimicrobial properties, Minimum inhibitory concentration

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ABSTRACT: In today's world, the medicine enterprise is among the quickest developing companies because of outbreaks of diverse sicknesses and antimicrobial opposition of diverse microbes towards typically utilized antibiotics. The control of diverse sicknesses and the traditional topic of treatment along with Ayurveda and home-based treatments also carries out a vital function within the part. Consequently, using an idea from home-based treatments and Ayurveda, these studies was created and done to show the anti-bacterial usage of Nyctanthes arbor-tristis leaves scientifically. A realize that is relative been achieved to judge the anti-bacterial pastime of *Nyctanthes arbor-tristis* towards *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* and *Salmonella typhi* from decided on leaves of *Nyctanthes arbor-tristis* from unique geographic places. The leaves of the plant are seen to function as the maximum green component with anti-bacterial pastime towards diverse pathogens analyzed. The anti-bacterial pastime of methanolic and chloroform extracts is analyzed with all the help of utilizing the disk diffusion technique. The ethanol extract of leaves has a greater pastime that is anti-bacterial chosen pathogenic microsystem when compared with the methanol and chloroform extract. The ethanol extracts of leaves are effective with anti-bacterial pastime towards all chosen microsystems. The leaves of *Nyctanthes arbor-tristis* have anti-bacterial, antifungal and anti-inflammatory properties that will be especially ideal for just about any microbial and condition that is fungal. Consequently, the leaves of *Nyctanthes arbor-tristis* are used to cope with diverse trivial mycosis and pyogenic transmissions and the enteric temperature. These relative studies is accomplished with all the help of utilizing acquiring the *Nyctanthes arbor-tristis* makes specimens from chosen places.

INTRODUCTION: Medicinal plant life has diverse antimicrobial marketers, which can be powerful towards a wide variety of microbes, that's why maximum medicinal plant life is broadly used for ailment control as an entire and, on occasion, the special components of the plant.

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Sometimes extracts of various solvents are organized to test the antimicrobial activity (anti-bacterial activity). Such medicinal plant life displays their significance due to the fact because of non-stop use of selective antimicrobial marketers, the microbes will be inclined to expand resistance residences of their unique due to the R-gene gift because the more chromosomal DNA of Bacteria and different microbes, in this case, maximum of the medical groups are searching out special medicinal plant life to collect special lively compounds and unique residences to create new composition and goal the diverse, unique components of microbes, mainly microorganism to

kill and inhibit the boom of micro organism to manipulate bacterial ailment. *Nyctanthes arbor-tristis*, additionally known as Harsingar and Parijat, belongs to the own circle of relatives Oleaceae. Various preceding investigations have proven that *Nyctanthes arbor-tristis* has anti-bacterial, antifungal, antiviral, analgesic, and antipyretic residences. These plant life additionally have antimalarial, antihelminthes and antiallergic residences. Several latest researches display that this plant additionally has anti-oxidant and liver-protecting residences. In this study work, the leaves of those plants that lives from special geographical places are accrued and examined for antimicrobial activity, mainly anti-bacterial residences towards *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* and *Salmonella typhi*.

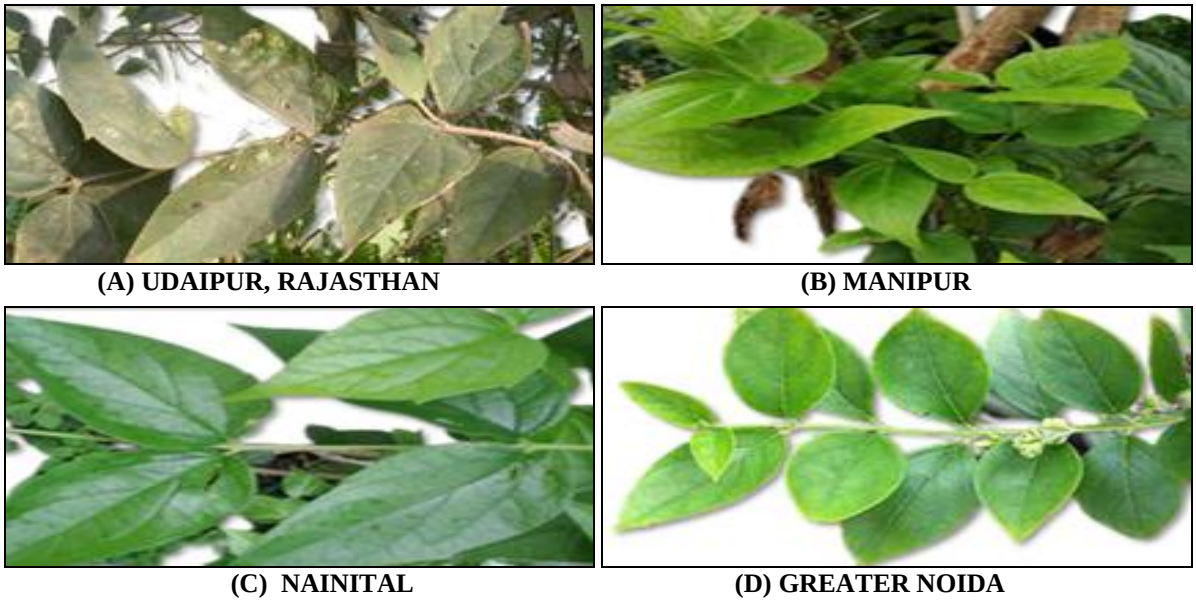
Nyctanthes arbor-tristis: *Nyctanthes arbor-tristis* is likewise known as night flowering jasmine, parijat, and shiuli. It belongs to the own circle of relatives of Oleaceae. This plant is observed in South Asia and Southeast Asia. This is a small tree that grows 10-30 ft lengthy. Their leaves are easy and contrary 6-12cm lengthy and 2-6. five cm extensive with the whole margin. This tree is on occasion known as the Tree of Sorrow because this plant's plant life loses its brightness at night. This plant has additionally cited its function and importance in our Veda. The leaves of this plant are utilized in ayurvedic remedies and homeopathy and domestic treatments to remedy ache and fever, and different infections. The leaves of this plant have numerous lively compounds like D-mannitol, β-Sitosterol, Flavanol Glycosides, Nicotiflorin, Oleanolic acid, Tannic acid, Ascorbic acid, Methyl salicylate, glycosides, carotene, glucose, and benzoic acid.

MATERIALS AND METHODS:

Sample Collection of Plants: Leaves of *Nyctanthes arbor-tristis* collected from Udaipur (Rajasthan), Manipur, Nainital (Uttarakhand), and Greater Noida (Gautam Budh Nagar, U.P.). All leaf specimens are shown in **Fig. 1**. In selected geographical locations, temperature, percentage of rain, and other environmental factors vary. The Collection details of leaves and their various conditions are shown below in **Table 1.**

TABLE 1: SHOWS THE VARIOUS GEOGRAPHICAL LOCATIONS ALONG WITH SAMPLE COLLECTION DATE, TEMPERATURE, AND RAINFALL PERCENTAGE

S. no.	Location	State	Collection Date	Temp.	Rainfall Percentage
1	Udaipur (Rajasthan)-RJ	Rajasthan	15-Mar-2021	24-29°C	25
2	Manipur-MR	Manipur	28-Mar-2021	16-25°C	64
3	Nainital (NT)	Uttarakhand	07-Oct-2019	08°-19°C	45
4	Greater Noida (GN)	Uttar Pradesh	17-Sep- 2019	27°-38C	15



(A) UDAIPUR, RAJASTHAN **(B) MANIPUR**

(C) NAINITAL **(D) GREATER NOIDA**

FIG. 1: LEAF SPECIMENS OF *NYCTANTHES ARBOR-TRISTIS* FROM DIFFERENT GEOGRAPHICAL LOCATIONS. WHERE A. SHOWS UDAIPUR, RAJASTHAN, B MANIPUR, C NAINITAL, AND D GREATER NOIDA

Specimen Validation and Authentication: The leaves of *Nyctanthes arbor-tristis* are validated and authenticated by Dr. Shailesh Solanki, Associate Professor, Department of Microbiology Faculty of Sciences, with Noida International University, U.P. with the authentication number NAT/006/2019 & NAT/239/2021. I am very thankful for this work.

Extract Preparation: *Nyctanthes arbor-tristis* leaves are gathered from chosen geographic locations, washed with distilled water, and permitted to dry in a heat oven in a closed chamber. After the drying procedure has been finished, the leaves are steamed in a mixer grinder to transform them to powder type. Now the two grams of dried powder is combined with 100 ml of ethanol, methanol, and chloroform and put in the shaker for 18-24 h. Following this, the crude extracts of ethanol, methanol, and chloroform are filtered through Whatman filter paper individually and permitted to evaporate. The different concentrations have been decided through the dilution method after the evaporation process. Because of this scholarly research, 5 dilutions in ascending purchase are prepared (0.25%, 0.050%, 1.5%, 4.5% and 13.5%).

Qualitative Phytochemical Tests: Different tests are conducted to check on the existence of anthraquinone, terpenoids, saponin, flavonoid, and glycosides according to try that is standard. The results of the many qualitative tests being phytochemical are mentioned in **Table 2.**

Anthraquinone test: 1 ml of extracts of each leaf specimen is boiled with 2 ml of sulfuric acid (H_2SO_4) and filtered when hot. Now, the filtrates are shaken with 2 ml of chloroform. The chloroform layer is carefully removed with Pipette and 2 ml of ammonia, and a color change is observed in blue or red, showing the presence of anthraquinone.

Test for Terpenoids: 1 ml of every leaf extract had been combined with 4 ml of chloroform. Now concentrate (H_2SO_4) 6 ml carefully to make a layer. The forming of a color that is reddish-brown the clear presence of terpenoids.

Saponin test: 0.5ml of each and every leaf extract is blended with 2 ml of distilled water in a test shake and pipe regularly to see mixture development. Now, the mixture is combined with 3 drops of essential olive oil and shakes regularly, and observes the synthesis of emulsion that indicates the formation of saponins.

Flavonoid test: 2 ml of diluted ammonia is added with all the extracts of different solvents from leaves, and today 1 ml of concentrated H_2SO_4 is added. Yellow formations that disappear with time show the presence of flavonoids.

Test for Glycosides: 100µl of plant test drawn in test tubes and add 2.0 ml then of chloroform then add 3.0 ml of concentrated H_2SO_4. Reddish-brown indicates the presence of terpenoids and greenish formation that is yellowish the presence of steroids.

Sample Selection of *Staphylococcus aureus, Streptococcus pyogens, Salmonella typhi,* and *Pseudomonas aeruginosa*: Bacterial samples of *Staphylococcus aureus (ATCC43300)* and *Streptococcus pyogens* (19615) are prepared by using culture and subculture techniques in nutrient broth, and nutrient agar from SRL Laboratories Noida, U.P. and *Salmonella typhi (ATCC 6539)* bacterial samples are obtained by growing microbes on XLD agar and *Pseudomonas aeruginosa (ATCC 15442)* are obtained by growing microbes on LB broth and agar, from SRL Laboratories Noida, U.P. Three commonly used antibiotics Streptomycin (X-GEN, 1000mg), Azithromycin (500mg) and Tetracyclin (200mg) are used as a Control for Antimicrobial Testing.

Inoculum Preparation: The bacterial strains of *Staphylococcus aureus* and *Streptococcus pyogens* are grown on nutrient agar and blood agar, *Salmonella typhi* on XLD agar, and *Pseudomonas aeruginosa* in LB broth and agar (Himedia, India) at 37 °C for 24-36 h. Stock is kept in the refrigerator at 4 °C.

Antimicrobial Testing: The antimicrobial activity of the ethanol, methanol, and chloroform extracts of the leaves of *Nyctanthes arbor-tristis* collected from different geographical locations is checked by the agar-disc diffusion method.

Minimum Inhibitory Concentrations: Minimum inhibitory concentrations of different extracts of leaves collected from different geographical regions are observed in plates surrounding the

wells' clear zone after incubation for the desired time. The zone of inhibition is measured in micrometers of selected pathogenic bacteria against the selected antibiotics as the control. The entire value of MIC is mentioned in **Table 3** along with the standard deviation, and the graphical representation of the MIC value of different geographical locations against different microbes is shown in Graph 1.

Statistical Analysis: All lab work is performed in triplicate during the work, and the data was recorded as means ± standard deviation (SD). A one-way ANOVA test is also performed on all statistical data where $p<0.5$ is taken as Significant.

RESULTS AND DISCUSSIONS:

Phytochemical Qualitative Assay: The dining table of phytochemical analysis obviously demonstrates anthraquinones exist in specimens of leaves gathered from Manipur and Nainital, where terpenoids, saponins, flavonoids, and glycosides are very nearly contained in each specimen of leaves and their extracts. Flavonoids are observed to become higher in ethanol than methanol plus in chloroform specimens. Saponins and glycosides can be found nearly in each test. The ethanol and methanol extracts of Manipur and Nainital (Uttarakhand) have the total amount that is greatest of phytochemical in comparison to Udaipur (Rajasthan) and better Noida (UP) that are suggested by **Table 2.** Anthraquinones are known antimicrobial agents which are additionally present in some edible flowers and have marked task that is anti-bacterial many germs; they disrupt the cell membrane layer of germs. Alongside terpenoids, flavonoids, saponins, and glycosides also provide antimicrobial activity by breaking and denaturing the fundamental substances of microbes, especially in germs.

TABLE 2: RESULTS OF QUALITATIVE ANALYSIS EXTRACTS OF ETHANOL, METHANOL AND CHLOROFORM *NYCTANTHES ARBOR-TRISTIS*

S. no.	Phytochemical tests	RJ-E	RJ-M	RJ-C	MR-E	MR-M	MR-C	NT-E	NT-M	NT-C	GN-E	GN-M	GN-C
1	Anthraquinones	-	-	-	+ +	+	-	+ +	+	-	-	-	-
2	Terpenoids	+ +	+	-	+ +	++	+	+ + +	+ +	+	+	+	-
3	Saponins	+ +	+	+	+ + +	+ +	+ +	+ + +	+ +	+	+ +	+	+
4	Flavonoids	+ +	+	-	+ + +	+ +	+	+ +	+ +	+	+ +	+	-
5	Glycosides	+ +	+	+	+ +	+ +	+	+ +	+ +	+	+ +	+ +	+

Table 2 – Represents the results of phytochemical analysis of the leaves of *Nyctanthes arbor-tristis* selected from Udaipur (Rajasthan), Manipur (M.P), Nainital (Uttarakhand), Greater Noida (Uttar Pradesh) where E-Ethanol, M-Methanol, C-Chloroform and RJ-Udaipur Rajasthan, MR-Manipur, NT-Nainital, GN- Greater Noida (UP). Symbols Show (-) absent ,(+) slightly present, (++) moderate present, (+++) highly present.

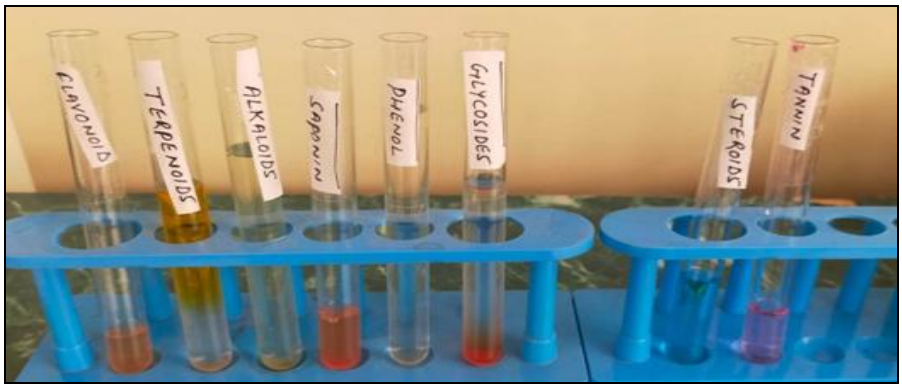


FIG. 2: PHYTOCHEMICAL QUALITATIVE RESULT OF VARIOUS TESTS

Agar Disc Diffusion Test: The anti-bacterial activity of *Nyctanthes arbor-tristis* leaves from different geographical locations is tested using the Kirby-Bauer disc diffusion method for this work; the prepared dilution of various leaf extracts in various concentrations (0.25%, 0.50%, 1.5%, 4.5%, and 13.5%) is soaked in a plain disc and applied as an antimicrobial agent for antimicrobial testing. A separate plate of Muller-Hinton agar is prepared for each extract with the different leaf samples for antimicrobial testing. Now, the bacterial sample is spread over the plate with the help of the sterile

cotton swab on the agar plates. Within the agar plates, the wells are prepared with the help of a tip and the soaked disc is loaded into each specific well for antimicrobial tastings. The plates are now incubated at 37 °C for 18-24 h. After the incubation period, the inhibition zone is measured in micrometers that appear around the disc.

Minimum Inhibitory Concentrations: After the dilution preparation and method selection, the extracts and their various concentrations are analyzed to check the minimum inhibitory concentration (MIC) of the lowest amount of extracts that inhibit the growth of selected bacteria. When the extracts are soaked in a plane disc and placed in a Petri plate along with the selected bacteria, the extracts produce the zone of inhibition around each well of antibiotics. The zone of inhibition is measured in micrometers and the lowest value of extracts showing the zone of inhibition is reported as the MIC value in **Table 3.** Activity of lower MIC value compared to standard antimicrobial agents. Streptomycin, Azithromycin, and Tetracyclin have a satisfactory performance high at 13.5 and low at 1.50 and 0.50 concentrations to show antimicrobial activity. In this study, the DNA of the bacterium and the cell membrane is targeted to determine the anti-bacterial activity of the extracts.

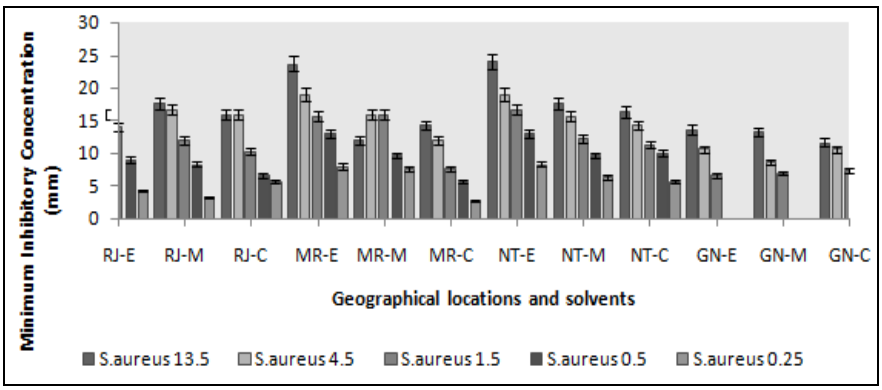
Extracts inhibit the production of DNA gyrase and topoisomerase from inhibiting DNA replication and binary fission and protein of the cell membrane from inhibiting the growth of microbes. Here, different extracts have different antimicrobial activity against selected microbes at different concentrations. While some can inhibit the growth of bacteria, some cannot inhibit bacterial growth.

TABLE 3: MINIMUM INHIBITORY CONCENTRATION OF ETHANOL, METHANOL AND CHLOROFORM EXTRACTS

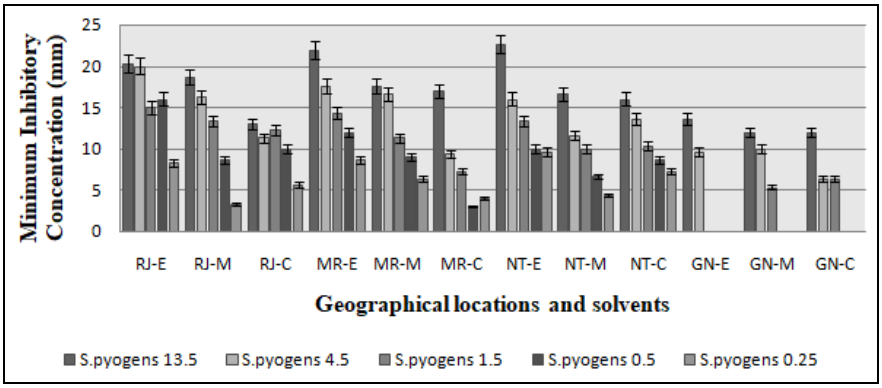
	Staphylococcus aureus	***Streptococcus pyogens***	***Salmonella typhi***	***Pseudomonas aeruginosa***
E/ Ab	**Concentrations of Extracts and Antibiotics in (mg/ml) : Zone of Inhibition ± Standard Deviation(mm)**			
RJ-E	13.5 : 19 ± 0.66	13.5 : 20.33 ± 2.51	13.5 : 20 ± 2	13.5 : 22.66 ± 4.16
	4.5 : 16 ± 0	4.5 : 20 ± 2	4.5 : 16 ± 2	4.5 : 15 ± 1
	1.5 : 14 ± 1	1.5 : 15 ± 1	1.5 : 14.33 ± 1.52	1.5 : 14 ± 3.60
	0.50 : 9 ± 1	0.50 : 16 ± 2	0.50 : 13.33 ± 1.15	0.50 : 11 ± 2.64
	0.25 : 4.33 ± 0.28	0.25 : 08.33 ± 2.08	0.25 :0 ± 0	0.25 : 0 ± 0
RJ-M	13.5 : 17.66 ± 1.52	13.5 : 18.66 ± 1.52	13.5 : 17.33 ± 2.08	13.5 : 13 ± 3
	4.5 : 16.66 ± 3.05	4.5 : 16.33 ± 2.51	4.5 : 15.33 ± 3.05	4.5 : 13.66 ± 4.50
	1.5 : 12 ± 2	1.5 : 13.33 ± 3.05	1.5 : 13.33 ± 3.51	1.5 : 11.33 ± 2.08
	0.50 : 08.33 ± 2.08	0.50 : 08.66 ± 1.52	0.50 : 10.66 ± 3.78	0.50 : 05 ± 0.45
	0.25: 03.33 ± 0.30	0.25 : 03.33 ± 0.30	0.25 : 07 : 030	0.25 : 04.33 ± 0.50
RJ-C	13.5 : 16 ± 1	13.5 : 13 ± 1	13.5 : 15.66 ± 1.52	13.5 : 14.66 ± 4.16
	4.5 : 16 ± 2	4.5 : 11.33 ± 9.86	4.5 : 14.66 ± 4.50	4.5 : 11.33 ± 3.21
	1.5 : 10:33 ± 0.57	1.5 : 12.33 ± 2.51	1.5 : 11 ± .60	1.5 : 07.66 ± 3.51
	0.50 : 06.66 ± 2.08	0.50 : 10 ± 1	0.50 : 09.33 ± 1.15	0.50 : 06.33 ± 2.51
	0.25 : 05.66 ± 4.93	0.25 : 05.66 ± 0.49	0.25 : 04.66 ± 0.50	0.25 : 06 ± 0.51
MR-E	13.5 : 23.66 ± 2.51	13.5 : 22 ± 2	13.5 : 17.33 ± 3.05	13.5 : 20.66 ± 5.50
	4.5 : 19 ± 1	4.5 : 17.66 ± 2.51	4.5 : 15.33 ± 4.04	4.5 : 20.66 ± 1.52
	1.5 : 15.66 ± 1.52	1.5 : 14.33 ± 4.50	1.5 : 12.33 ± 4.93	1.5 : 16.66 ± 5.85
	0.50 : 13 ± 2.64	0.50 : 12 ± 3.60	0.50 : 11.33 ± 2.51	0.50 : 08 ± 1
	0.25 : 08 ± 0.70	0.25 : 08.66 ± 0.76	0.25 : 05.66 ± 0.49	0.25 : 07.66 ± 1.52
MR-M	13.5 : 12 ± 2	13.5 : 17.66 ± 2.51	13.5 : 20 ± 1.73	13.5 : 18.33 ± 2.08
	4.5 : 16 ± 4.35	4.5 : 16.66 ± 5.85	4.5 : 19 ± 1	4.5 : 16.33 ± 2.51
	1.5 : 16 ± 2	1.5 : 11.33 ± 4.93	1.5 : 12.33 ± 4.93	1.5 : 09 ± 1
	0.50 : 9.66 ± 0.57	0.50 : 09 ± 1	0.50 : 11.33 ± 3.21	0.50 : 05 ± 0.45
	0.25 : 07.66 ± 1.52	0.25 : 06.33 ± 5.50	0.25 : 05 ± 45	0.25 : 03 ± 0.51
MR-C	13.5 : 14.33 ± 3.78	13.5 : 14 ± 3.60	13.5 : 14 ± 3.60	13.5 : 13.66 ± 2.51
	4.5 : 12 ± 4.35	4.5 : 09.33 ± 1.52	4.5 : 11.66 ± 4.72	4.5 : 11.66 ± 4.04
	1.5 : 07.66 ± 1.52	1.5 : 07.20 ± 0.64	1.5 : 07.66 ± 2.51	1.5 : 08 ± 1.73
	0.50 : 05.66 ± 0.49	0.50 : 03 ± 0.51	0.50 : 04.66 ± 0.50	0.50 : 06.33 ± 0.63
	0.25 : 02.66 ± 0.46	0.25 : 04 ± 0.40	0.25 : 06.33 ± 2.30	0.25 : 07.33 ± 2.08
NT-E	13.5 : 24 ± 4	13.5 : 22.66 ± 5.03	13.5 : 22.66 ± 3.05	13.5 : 16 ± 2
	4.5 : 19 ± 1	4.5 : 16 ± 3	4.5 : 18.33 ± 2.08	4.5 : 16 ± 5.56
	1.5 : 16.66 ± 2.08	1.5 : 13.33 ± 3.51	1.5 : 16.66 ± 1.52	1.5 : 11.66 ± 10.69

	0.50 : 13 ± 5.19	0.50 : 10 ± 0.50	0.50 : 13.33 ± 3.05	0.50 : 06.66 ± 5.85
	0.25 : 08.33 ± 7.37	0.25 : 09.66 ± 2.08	0.25 : 08.66 ± 2.51	0.25 : 06.33 ± 2.51
NT-M	13.5 : 17.66 ± 2.51	13.5 : 16.66 ± 3.08	13.5 : 16.66 ± 3.15	13.5 : 16.33 ± 152
	4.5 : 15.66 ± 1.52	4.5 : 11.66 ± 1.21	4.5 : 14 ± 2.64	4.5 : 16 ± 2
	1.5 : 12.33 ± 4.95	1.5 : 10 ± 1	1.5 : 6 ± 5.29	1.5 : 11.66 ± 5.85
	0.50 : 9.66 ± 4.04	0.50 : 6.66 ± 5.85	0.50 : 9 ± 2	0.50 : 7.66 ± 3.21
	0.25: 6.33 ± 5.68	0.25: 4.33 ± 3.78	0.25 : 7.33 ± 4.04	0.25 : 5.33 ± 4.61
NT-C	13.5 : 16.33 ± 1.52	13.5 : 16 ± 1.73	13.5 : 10.33 ± 3.21	13.5 : 14.66 ± 6.02
	4.5 : 14.33 ± 3.78	4.5 : 13.66 ± 4.04	4.5 : 11.66 ± 2.08	4.5 : 14.33 ± 3.78
	1.5 : 11.33 ± 2.51	1.5 : 10.33 ± 0.57	1.5 : 8 ± 0.96	1.5 : 11.33 ± 9.86
	0.50 : 10 ± 1	0.50 : 8.66 ± 0.57	0.50 : 9.16 ± 0.76	0.50 : 6.66 ± 1.15
	0.25 : 5.66 ± 4.96	0.25 : 7.33 ± 2.08	0.25 : 6 ± 0.60	0.25 : 4 ± 4
GN-E	13.5 : 13.66 ± 3.21	13.5 : 13.66 ± 3.51	13.5 : 14 ± 3.74	13.5 : 13.33 ± 3.05
	4.5 : 10.66 ± 2.88	4.5 : 9.66 ± 4.04	4.5 : 8 ± 5.29	4.5 : 6.66 ± 2.80
	1.5 : 6.66 ± 3.05	1.5 : 0 ± 0	1.5 : 3 ± 0.35	1.5 : 6.66 ± 2.08
	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0
	0.25 : 0 ± 0	0.25: 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0
GN-M	13.5 : 13.33 ± 3.05	13.5 : 12 ± 2.64	13.5 : 13 ± 1	13.5 : 12 ± 0
	4.5 : 8.66 ± 3.05	4.5 : 10 ± 2	4.5 : 10.66 ± 1.15	4.5 : 10.66 ± 2.08
	1.5 : 7 ± 3	1.5 : 5.3 ± 0.35	1.5 : 8.66 ± 1.52	1.5 : 10 ± 1
	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0
	0.25 : 0 ± 0	0.25: 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0
GN-C	13.5 : 11.66 ± 1.52	13.5 : 12 ± 1	13.5 : 10 ± 2	13.5 : 10.33 ± 1.52
	4.5 : 10.66 ± 1.15	4.5 : 6.33 ± 5.50	4.5 : 8.33 ± 1.15	4.5 : 8.66 ± 4.50
	1.5 : 7.33 ± 3.78	1.5 : 6.33 ± 2.51	1.5 : 6 ± 3.60	1.5 : 6.33 ± 5.50
	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0
	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0
SM	13.5 : 11.33 ± 1.52	13.5 : 10.33± 3.21	13.5 : 11.33 ± 3.21	13.5 : 13.66 ± 3.51
	4.5 : 13.33 ± 2.08	4.5 : 9.33 ± 2.08	4.5 : 13 ± 4.35	4.5 : 10 ± 1.00
	1.5 : 0 ± 0	1.5 : 0 ± 0	1.5 : 0 ± 0	1.5 : 0 ± 0
	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0
	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0
AZ	13.5 : 15.33 ± 4.72	13.5 : 24.33± 4.50	13.5 : 15.66 ± 1.52	13.5 : 14 ± 1
	4.5 : 10.66 ± 2.08	4.5 :13 ± 2.64	4.5 : 11 ± 4.35	4.5 : 9.33 ± 0.90
	1.5 : 0 ± 0	1.5 : 0 ± 0	1.5 : 0 ± 0	1.5 : 0 ± 0
	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0
	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0
TC	13.5 : 16.66 ± 2.08	13.5 : 14.33± 5.13	13.5 : 13.33 ± 3.51	13.5 : 13.33 ± 3.05
	4.5 : 11.66 ± 2.08	4.5 : 13.66 ± 2.51	4.5 : 11.66 ± 2.08	4.5 : 10 ± 1.00
	1.5 : 0 ± 0	1.5 : 0 ± 0	1.5 : 0 ± 0	1.5 : 0 ± 0
	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0	0.50 : 0 ± 0
	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0	0.25 : 0 ± 0

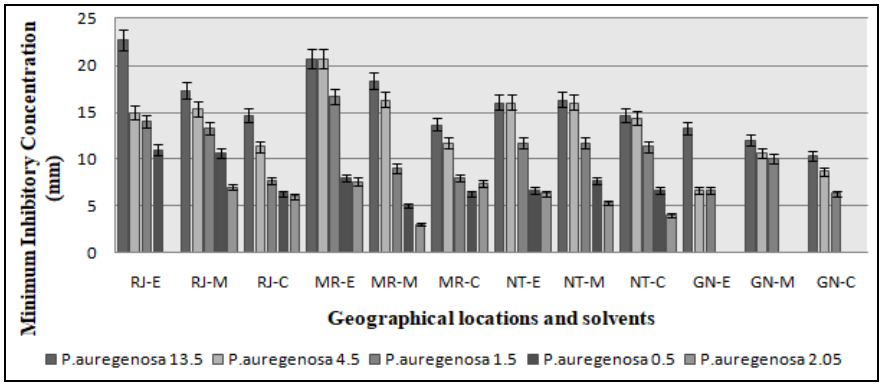
Table 3 shows the inhibition distance zone (in mm) of *Nyctanthes arbor-tristis* extracts of ethanol, methanol, and chloroform and standard anti-bacterial agents against the selected bacteria. E-Extracts, Ab-Antibiotics, E-Ethanol, M-Methanol, C-Chloroform and RJ-Rajasthan, MA-Manipur, NT-Nainital, GN- Greater Noida, Sm-Streptomycin, Az-Azithromycin, Tc-Tetracyclin.



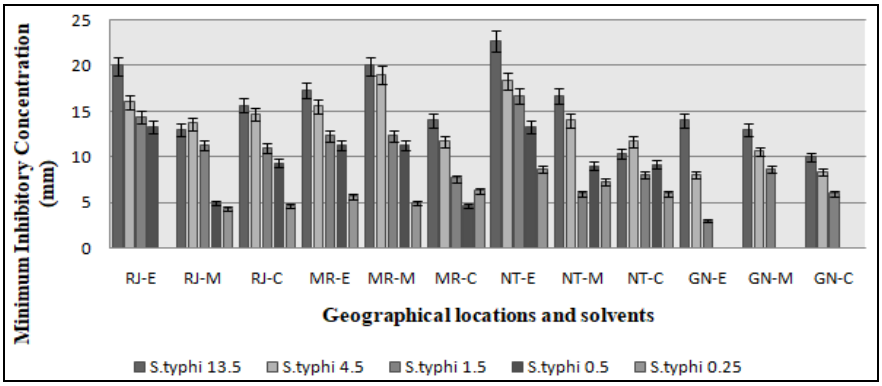
STAPHYLOCOCCUS AUREUS



STREPTOCOCCUS PYOGENS



PSEUDOMONAS AERUGINOSA



SALMONELLA TYPHI

GRAPH 1: SHOWS THE COMPARATIVE STUDY OF PAPAYA EXTRACTS OF ETHANOL, METHANOL, AND CHLOROFORM OF DIFFERENT SELECTED GEOGRAPHICAL LOCATIONS AGAINST *S. AUREUS, S.PYOGENS, S.TYPHI* AND *P.AERUGINOSA*

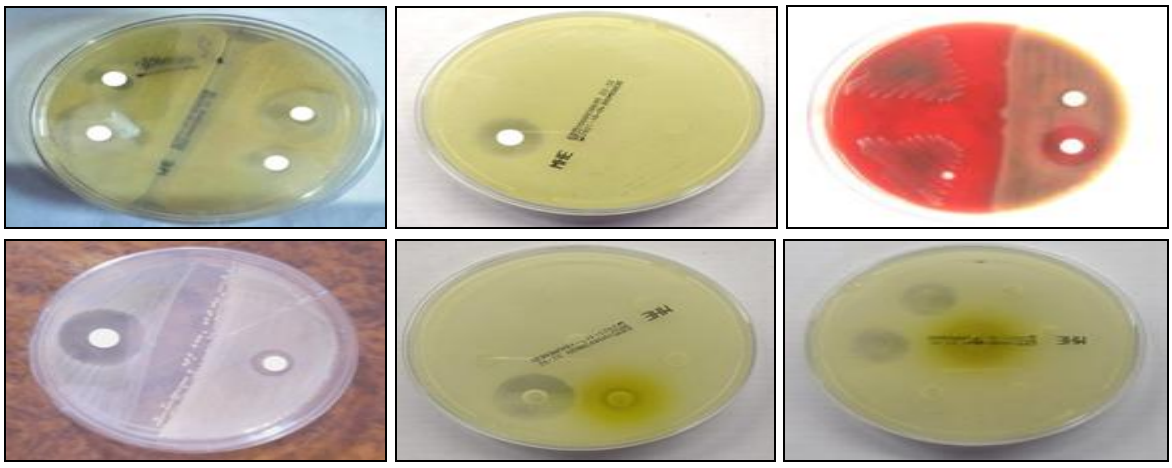


FIG. 3: SHOWING THE INHIBITION ZONE AGAINST THE SELECTED BACTERIA ALONG WITH THE DIFFERENT CONCENTRATIONS OF *NYCTANTHES ARBOR –TRISTIS* LEAVES

CONCLUSIONS: The leaves of varied *Nyctanthes arbor-trisitis* from various geographic places are tested along with chosen pathogenic germs such as for example, *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa* and *Salmonella typhi* in our research. Ethanol extracts have significantly more activity that is anti-bacterial methanol and chloroform extracts.

Phytochemical activity implies that the leaves of *Nyctanthes arbor-tristis* have actually flavonids, terpenoids, saponins and glycosides within the extracts regarding the leaves. Anthraquinones are somewhat contained in some extracts. Existence of those metabolites being secondary active substances) in leaves of *Nyctanthes arbor-tristis* with every solvent shown in **Table 2.** These metabolites work well against many germs that can be pathogenic with chosen germs such as *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa,* and *Salmonella typhi.*

Finally, the research demonstrates that *Nyctanthes arbor-tristis* leaves from various areas are geographic anti-bacterial tasks against chosen germs. An optimum anti-bacterial task reported with ethanol extracts from Manipur and Nainital compared with Udaipur and better Noida showed in **Tables 3** and **Graph 1.**

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Ethics Statement: This study does not contain studies with human participants or animals performed by any authors. All research work is conducted in a suitable scientific manner and does not harm or affect human populations.

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CONFLICT OF INTEREST: I declare that there is no conflict of interest.

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