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EXTRACTION AND FRACTIONATION OF DATURA METEL LINN PHYTOCONSTITUENTS AND THEIR EVALUATION AS ANTIBACTERIAL COMPOUNDS

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ABSTRACT

The pharmacological properties of *Datura metel Linn* are well known. The current study aimed to investigate the antibacterial activity of methanol extract and fractions of *D. metel L*. leaves. The presence of alkaloids, flavonoids, saponins, and steroids is revealed by a primarily qualitative analysis of phytochemicals. Using the agar well diffusion method, MIC, and MBC, antibacterial activity against human pathogens such as *E. coli, S. enterica typhimurium, S. flexneri*, and *S. aureus* revealed the presence of phytochemicals responsible for the killing of pathogens. Methanol extract fractions also had significant antibacterial activity.

Keywords: *Datura metel Linn*, Phytoconstituents, Antibacterial agent, Fractionation

INTRODUCTION

Herbs were used in healing rituals by indigenous cultures such as Rome, Egypt, Iran, Africa, and America, while others developed traditional medical systems such as Unani, Ayurveda, and Chinese Medicine in which herbal therapies were used systematically **[1]**. plant-based antimicrobials have immense potential to combat bacterial, fungal, protozoal, and viral diseases without any known side effects **[2]**. Most countries now use traditional medicinal systems that include the use of herbal drugs and remedies. Many have incorporated plants as a source of medicinal agents into their primary modality of health care **[3, 4]**.

D. metel has been found to contain a wide range of phytochemicals. These

phytoconstituents comprise alkaloids, flavonoids, phenols, tannins, saponins, and sterols. The phytoconstituents of Datura were analyzed from various parts of the plant like the leaf **[5, 6]** root **[7]**, and shoot **[8-10]**. D. metel L contains tropane alkaloids which are used as a sedative, anti-spasmodic, and mydriatic agents **[11]**. The whole plant, but especially the leaves and seed, has anesthetic, a hallucinogenic, anti-asthmatic, anti-spasmodic, anti-tussive, narcotic, bronchodilator, anodyne, hypnotic, and mydriatic effects. Leaves are used as a local application for rheumatic swellings of the joints, Lumbago, Sciatica, Neuralgia, painful Tumours, Scabies, Eczema, Allergy, and glandular Inflammations, such as Mumps; used externally for earache and smoked to relieve spasmodic Asthma. Seeds are also used externally for piles **[12]**. Various solvent extracts of D. metel were found to have documented antibacterial, antifungal, and anti-inflammatory properties **[13, 14]**.

Different solvent compositions based on the nature of compounds to be separated and isolated are used in column chromatography, it is the most effective technique used in the separation of plant extract into its components in its pure form **[15]**. The fractions of the flowers of *D. metel L* led to the isolation of a new compound named jangijnhualine A, and five known megastigmane sesquiterpenes through separated silica gel & ODS column chromatography **[16]**.

The present study aims to isolate the fractions of methanol extract from leaves of *D. metel L.* and to investigate their effect on human pathogens.

MATERIALS AND METHODS

Collection and processing of the plant

D. metel L. was collected near the Christ College campus in Rajkot. Dr. Jignasa Joshi, an Assistant Professor in the Department of Biology at Christ College in Rajkot, identified all of the plants. The leaves were separated and air-dried for several days. A mechanical grinder was used to powder dried leaves. The powder was stored in an airtight bottle for future use **[17]**.

Preparation of Extract

Methanol was used in the extraction process. The extract was made by dissolving 100 grams of powder from *D. metel L.* leaves in 300 ml of methanol. For three days, this mixture was shaken on an orbital shaker at 40 rpm. After three days, the mixture was filtered through No. 1 Whatman filter paper. Air drying was used to allow the filtrate to evaporate. The extract was kept in the fridge for future use **[18]**. The extract stock solution was made by dissolving 100 milligrams of extract in 100 milliliters of dimethyl sulfoxide (DMSO) **[19]**. The stock solution was kept in the fridge at 4^{o}C. The extraction yield of

selected plants was calculated using the equation **[20]**.

The Yield. % = X1 x 100/X0

Where X1 denotes the weight of the extract after solvent evaporation and X0 denotes the dry weight of the plant powder before extraction.

Phytochemical analysis

Phytochemical tests were carried out to analyze the various chemical compounds present in the extract using the method described by Trease G.E **[21]**. **Steroid Screening**: Two milliliters of extract were dissolved in chloroform, and two milliliters of concentrated sulphuric acid were added to the mixture. The presence of steroids is indicated by the formation of red colour. **Glycoside testing**: Two milliliters of extract were dissolved in chloroform, and two milliliters of acetic acid were added to the mixture. After cooling the solutions, a few drops of sulphuric acid were added. A change in color from blue to green indicates the presence of Glycosides. **Flavonoids** were detected by adding 1ml of 10% lead acetate to 1ml of extract; the formation of a yellow precipitate indicates the presence of flavonoids. **Saponins** were determined by vigorously shaking two milliliters of the extract with five milliliters of distilled water and warming it. The stable foam formed indicated the presence of saponins. **Tannin testing:** 2 mL of the extract is thoroughly mixed with 2 mL of distilled water. The presence of tannins was revealed by the formation of green precipitate after the addition of a few drops of ferric chloride. **Amino acid analysis**: A few drops of ninhydrin reagent were added to one milliliter of extract. The purple color of the mixture indicates the presence of amino acids. **To test for alkaloids**, three milliliters of extract were mixed with one percent HCL and placed in a steam bath**.** A few drops of Mayer & Wagner's reagent were added to the mixture Turbidity indicates the presence of alkaloids.

Selection of Bacterial cultures

Bacteria purchased from MTCC Chandigarh included *Escherichia coli* (MTCC 443), *Salmonella enterica typhimurium* (MTCC 98), *Shigella flexneri* (MTCC1457), and *Staphylococcus aureus* (MTCC 3160). For later use, these bacteria were stored on N-Agar slant (Hi-media) at 4ºC.

Preparation of Inoculum

All of the bacteria were pre-cultured in Mueller Hinton broth (MHB) for 24 hours in a rotary shaker at 37°C. Following that, each strain was adjusted to a concentration of 10^8 cells/ml using the 0.5 McFarland standard **[22]**.

Antimicrobial analysis

The Agar well diffusion technique was used to measure antibacterial activity. Bacterial cultures were grown in N broth for 18 hours at 37ºC. Mueller-Hinton agar

(Himedia) plates were prepared and allowed to solidify. Using a sterile swab, bacteria were inoculated on plates. A well has been prepared using a sterilized cup-borer with a diameter of 8 mm. In the well, 0.1 ml of extract dissolved in DMSO was poured. For 24 hours, plates were incubated at 37°C in an incubator. Zones of inhibition were observed and measured in millimetres after 24 hours **[23]**.

Minimum inhibitory concentration (MIC)

The tube method was used to determine the minimum inhibitory concentration (MIC) of the extract. All tubes received 4ml of Mueller Hinton broth. 4ml of extracts was added to all the tubes except positive control. The mixture was thoroughly mixed. 4ml of the mixture was taken from the first tube and placed in the second. It should be noted that the first tube contained 100mg/ml of extract, and the concentration in the subsequent tube was half that of the previous tube. Similarly, the other ten tubes are set up for a twofold serial dilution. Except for the negative control, each tube received 0.1ml of pure microorganism culture. For 24 hours, all tubes were incubated at 37°C in an incubator. After 24 hours, visible bacterial growth was observed in the tubes. The MIC of that extract is the lowest concentration at which no bacterial growth is observed **[24]**. All assays were carried out in triplicate. DMSO was used as a control, and Levofloxacin was the standard drug.

Minimum bactericidal concentration (MBC)

A loop of culture from the MIC tube is streaked on the Mueller Hinton agar media plate and incubated at 37°C for 24 hours. After incubation, the plates were checked for bacterial growth **[25]**. The absence of growth in the plates indicates that the extract concentration is both bacteriostatic and bactericidal.

Column chromatography

Column chromatography is a separation technique in which a component of a mixture is separated by passing it through a glass column packed with the stationary phase and mobile phase. For the stationary phase, a slurry of silica gel is prepared in hexane. The slurry occupies two-thirds of the column (silica gel- 60 to 120 mesh). A mobile phase (liquid solvent) is added from the top of the column and flows down the column due to gravity. Following column preparation, the sample is loaded onto the column's stationary phase. The liquid solvent (mobile phase) hexane, ethyl acetate, and methanol in the various ratio is allowed to flow down through the column. The column elution began with 100 percent hexane and was then increased in polarity with ethyl acetate and methanol. The hexane: ethyl acetate ratio was 100 percent hexane, 10:1, 5:1, 1:1, 1:5, 1:10, and the

ethyl acetate: methanol ratio was 100 percent ethyl acetate, 10:1, 5:1, 1:1, 1:5, 1:10, and 100 percent methanol. The solvent (mobile phase) flow rate is kept constant at 1ml/min. Every 30 minutes, the separated compound is collected as fractions. This technique is used to separate chemicals from a mixture. Individual fractions are further investigated for structural elucidation **[26, 27]**.

RESULT AND DISCUSSION

Percentage yield extract

In the methanol extract of the leaves of *D metel L*. the percentage yield of the extract was found to be 5.0%. The number and quantity of phytochemicals present in the extract will determine the bioactivity including the antibacterial activity of the extract. More the yield of the extract may give more antibacterial activity but there may be a contradiction as the compounds present may or may not be antimicrobial agents.

Phytochemical analysis

Phytochemical analysis of the methanol extract of the leaves of *D. metel L.* showed the presence of Alkaloids, Flavonoids, Steroids, and Saponins as tabulated in **Table 1**. Other studies also support the result of this study **[28]**. Alkaloids, flavonoids, steroids, and saponins have antimicrobial properties **[29]**. Thus, the presence of these compounds in the extract signifies the antibacterial activity of the extract.

Antibacterial activities

Antibacterial activity of methanol extract from the leaves of *D. metel L.* against selected bacteria showed in **Table 2**. Antibacterial activity of this extract was found against all the selected bacteria. Maximum activity was against *S. enterica typhimurium and S. aureus* with the zone of inhibition of 14.0 ± 0.65 mm and 14.0 ± 0.36 mm respectively. While the least activity was against *E. coli* with a zone of inhibition of 10.0 ±0.98mm. Thus, both gram-positive and gram-negative bacteria were affected equally. Thus, this extract has a broad spectrum of antimicrobial activity.

Minimum Inhibitory Concentration

MIC of *D. metel L.* leaves extract prepared in methanol was 25 mg/ml for *S. aureus*, while it was 50 mg/ml for *E. coli* and *S. enterica typhimurium* and 100 mg/ml for *S. flexneri* which is tabulated in **Table 3**. MIC values indicated the amount of extract required for inhibiting the microorganisms. When compared with the standard antibiotic Levofloxacin concentration of extract required to inhibit the bacteria was more while the control did not show any antibacterial activity.

MINIMUM BACTERICIDAL CONCENTRATION

Methanol extract of leaves of *D. metel L.* showed MBC of 50 mg/ml for *E. coli* and

S. enterica typhimurium, 25mg/ml against *S. aureus* while 100 mg/ml for *S. flexneri* as shown in **Figure 1** which is the same as MIC. As a result, the MIC and MBC values are the same. A chemical is bactericidal if the MBC/MIC ratio is less than or equal to 4.0, and bacteriostatic if the MBC/MIC ratio is greater than 4.0, according to Konate **[30]**. As a result, the extract obtained was bactericidal, with MIC values equal to MBC.

FRACTIONATION OF EXTRACT BY COLUMN CHROMATOGRAPHY

Methanol extract from leaves of *D. metel L.* was taken for column chromatography. Various solvents like Hexane, Ethyl acetate, and Methanol were used for the elution of the fractions. 59 fractions were collected from the silica gel (60 – 120 mesh) for *D. metel L.* at the interval of 30 minutes with a flow rate of 1 ml/min is represented in **Table 4**.

Antimicrobial activity of fractions obtained from Column Chromatography

The fractions obtained from Column chromatography of methanol extract of *D. metel L.* were analyzed for antibacterial activity against *E. coli, S. aureus, S. flexneri, and S. enterica typhimurium* were shown in **Figure 2**.

Figure 2 reveals that all the fractions did not show antibacterial activity. 36th fraction showed maximum antibacterial activity against most of the selected bacteria. Except for fractions 7, 8, 36, 38, and 43, no fraction showed antibacterial activity against more than two bacteria. Many fractions showed antibacterial activity against single bacterial *S. aureus* only, while many fractions did not show any antibacterial activity. Fraction numbers 1 to 11 except fraction 3 showed significant antibacterial activity against *E. coli, S. aureus, and S. flexneri* but none of the fractions displayed antibacterial activity against *S. enterica typhimurium*. Fraction number 36 showed maximum antibacterial activity 14±0.46, 9.3±0.15, and 11±0.17 mm zone of inhibition against *E. coli, S. aureus, and S. flexneri*. The fractions which were eluted by polar solvent showed maximum activity. Hence the antimicrobial substances may be polar.

Table 1: Preliminary phytochemical evaluation of methanol extracts leaves of *D. metel L.*

Phytochemical constituents	Result
Alkaloids	+
Flavonoids	+
Saponins	+
Steroids	+
Tannins	-
Glycosides	-
Amino acids	-

(+) = present; (-) = absent

Table 2: Antibacterial evaluation of methanol extracts leaves of *Datura metel L.*

Bacteria	Mean Zone of inhibition ± SD (in mm)
E. coli	10.0 ±0.98
S. enterica typhimurium	14.0 ± 0.65
S. flexneri	10.5 ± 0.35
S. aureus	14.0 ± 0.36

Mean value of triplicate ± standard deviation

Table 3: Minimum Inhibitory Concentration of methanol extract of leaves of *D. metel L.* against selected bacteria

Organisms	Mean Minimum inhibitory concentration ± SD (mg/ml)		
	methanolic extract of leaves of *D. metel L*	DMSO as a control	Levofloxacin as standard
E. coli	50 ± 0	0	0.025 ± 0
S. enterica typhimurium	50 ± 0	0	0.025 ± 0
S. flexneri	100 ± 0	0	0.025 ± 0
S. aureus	25 ± 0	0	0.025 ± 0

Mean value of triplicate ± standard deviation

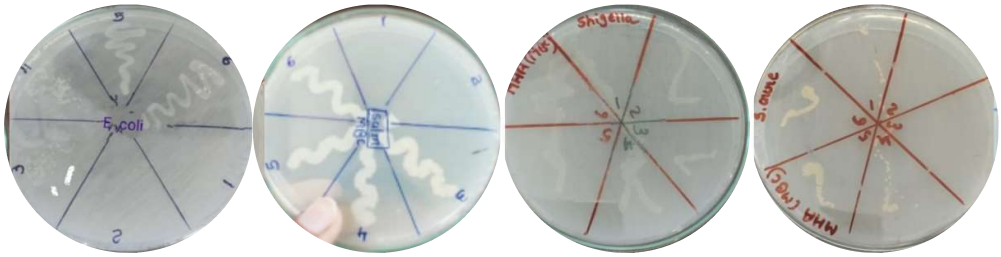

Figure 1: Minimum Bactericidal concentration of methanol extract of leaves of *D. metel L*

Table 4: Number of fractions obtained from methanolic extract of leaves of *D. metel L.* by column chromatography

Solvent system	Ratio	Volume (ml)	No. of Fractions
Hexane	100%	50	1
Hexane: Ethyl acetate	10:1	50	2
Hexane: Ethyl acetate	5:1	48	2
Hexane: Ethyl acetate	1:1	50	3
Hexane: Ethyl acetate	1:5	48	7
Hexane: Ethyl acetate	1:10	50	5
Ethyl acetate	100%	50	5
Ethyl acetate: Methanol	10:1	50	8
Ethyl acetate: Methanol	5:1	48	6
Ethyl acetate: Methanol	1:1	50	5
Ethyl acetate: Methanol	1:5	48	7
Ethyl acetate: Methanol	1:10	50	6
Methanol	100%	50	2

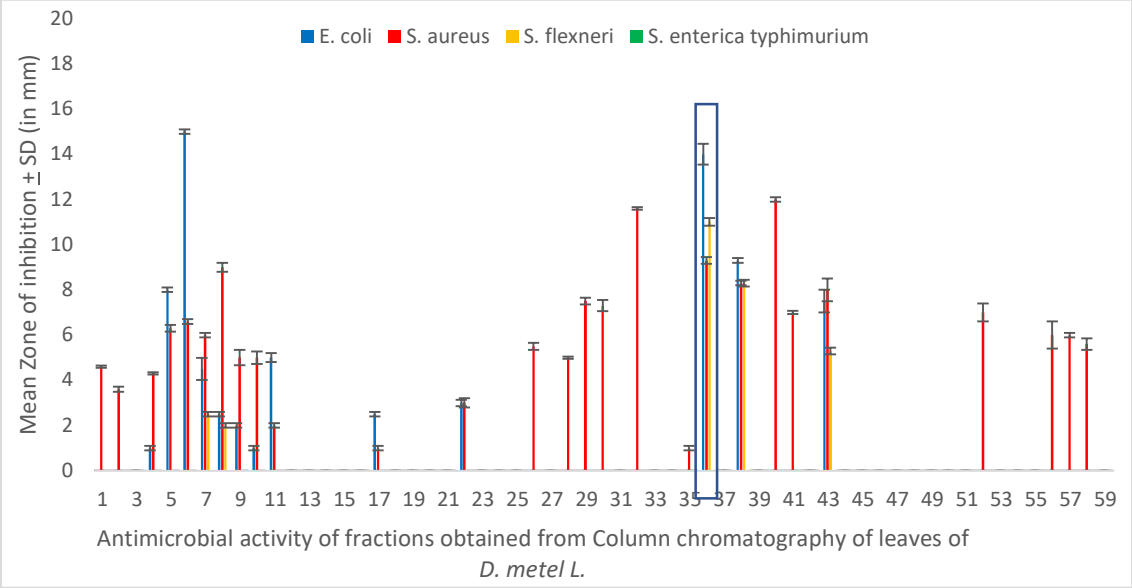

Figure 2: Antimicrobial activity of fractions obtained from Column Chromatography of methanol extract of leaf of *D. metel L.* against selected bacteria

CONCLUSION

In the present study, the fractions from methanol extract of leaves of *D. metel L.* were isolated by column chromatography to study their antibacterial activity. Methanol extract of leaves of *D. metel L.* showed significant antibacterial activity against all the selected bacteria. 36th fraction obtained by the polar solvents showed the maximum antibacterial activity against almost all the selected bacteria. Thus, the compounds present were polar as they were eluted by polar solvent. Most of the fractions showed antibacterial activity against gram-positive bacteria *S. aureus*. Further, this fraction can be used to identify the presence of compounds by various analytical techniques.

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Conflicts of Interest

The authors declare no conflict of interest

REFERENCES

[1] Ajeet Singh and Navneet. A review of Uttarakhand's medicinal plants and herbs (India): its traditional, ethanol-medicinal and antimicrobial potential. Nat Sci 2016;14(12):90-107]. ISSN 1545-0740 (print); ISSN 2375-7167 (online). http://www.sciencepub.net/nature. 16.doi:10.7537/marsnsj141216.16.

[2] Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. *Plants*. 2017; 6(2):16. https://doi.org/10.3390/plants6020016

[3] K.M. Manas, B. Pratyusha, N. Debj ani Phytochemicals–biomolecules for prevention and treatment of human diseases a review Int J Sci Eng Res, 3 (7) (2012), pp. 1-32

[4] C. Imo, F.O. Uhegbu Phytochemical analysis of Gongronema latifolium benth leaf using gas chromatographic flame ionization detector Int J Chem Biomol Sci, 1 (2) (2015), pp. 60-68

[5] Dhiman Anju, Lal Ratan. Phytochemical and Pharmacological status of Datura fastuosa Linn. International Journal of Research in Ayurveda and Pharmacy 2011; 2(1): 145-150.

[6] Donatus Ebere Okwu, Ephraim ChintuaIgara. Isolation, characterization, and antibacterial activity of alkaloid from Datura metel Linn leaves. African Journal of Pharmacy and Pharmacology 2009; 3(5): 277-281.

[7] Jamdhade1 MS, Survase SA, Kare MA, Bhuktar AS. Phytochemical Studies on Datura Metel Linn. In Marathwada region, Maharashtra. Journal of Phytology 2010; 2(12): 46-48.

[8] Akharaiyi FC. Antibacterial, Phytochemical and Antioxidant activities of Datura metel. International Journal of PharmTech Research 2011; 3(1): 478-483.

[9] John De Britto A, Herin Sheeba G r a c e l i n D. Datura metel Linn. - A plant with potential as antibacterial agent. International Journal of Applied Biology and Pharmaceutical Technology 2011a; 2(2): 429-433.

[10] Arshad Javaid, Sobiya Shafique, Shazia Shafique. Herbicidal Activity of *Datura Metel* L. against Phalaris minor Retz. Pak. J.Weed Sci. Res 2008; 14(3-4): 209-220.

[11] H. Nuhu. Alkaloid content of the leaves of three Nigerian Datura species. Nig J Nat Prod Med, 6 (2002), pp. 15-18

[12] M. Yusuf, J. Begum, M.N. Hoque, J.U. Chowdhury. Medicinal Plants of Bangladesh BCSIR, Chittagong, Bangladesh (2009), p. 794

[13] T. H. A. Alabri, A. H. S. Al Musalami, M. A. Hossain, A. M. Weli, and Q. Al-Riyami, "Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of Datura metel L," Journal of King Saud University, vol. 26, no. 3, pp. 237–243, 2014.

[14] F. C. Akharaiyi, "Antibacterial, phytochemical and antioxidant activities of Datura metel," International Journal of PharmTech Research, vol. 3, no. 1, pp. 478–483, 2011.

[15] Singh, Dr. Monika & Patra, Sukanya & Singh, Rajesh. (2021). Common techniques and methods for screening of natural products for developing of anticancer drugs. 10.1016/B978-0-12-821710-8.00015-1.

[16] Hx., Yang, By., Xia, Yg. *et al*. Chemical constituents from the flower of Datura metel L. Arch. Pharm. Res. 31, 1094–1097 (2008). https://doi.org/10.1007/s12272-001-1274-6

[17] P. Mozhiyarasi and R. Anuradha. Study on phytochemical analysis and antimicrobial activity of *Hyptis suaveolens* (L.) poit. Journal of Chemical and Pharmaceutical Research, 2016, 8(6):438-442

[18] Handa, S., Khanuja, S.P., Longo, G. and Rakesh, D.D. (2008) Extraction Technologies for Medicinal and Aromatic Plants. United Nations Industrial Development Organization and the International Centre for Science and High Technology, 260 p.

[19] Gadiyar A, Ankola AV, Rajpurohit L. Evaluation of the antimicrobial activity of *Ocimum sanctum* L. (tulsi) extract against *Streptococcus mutans* and *Lactobacillus acidophilus* - An in vitro study. Int J Health Sci Res. 2017; 7(4):224-228.

[20] Felhi, S., Daoud, A., Hajlaoui, H., Mnafgui, K., Gharsallah, N., and Kadri, A. (2017). Solvent extraction effects on phytochemical constituents profiles, antioxidant and antimicrobial activities and functional group analysis of *Ecballium elaterium* seeds and peels fruits. J. Food Sci. Technol. 37, 483–492. doi: 10.1590/1678-457x.23516.

[21] Trease G.E. and Evans W.C., Pharmacognosy, 11th edn.,

Bailliere Tindall, London, 45-50 (1989).

[22] Bhalodia, N. R., and Shukla, V. J. (2011). Antibacterial and antifungal activities from leaf extracts of *Cassia fistula* l.: an ethnomedicinal plant. J. Adv. Pharm. Technol. Res. 2, 104–109. doi: 10.4103/2231-4040.82956

[23] Valgas C., De Souza S.M., Smânia E.F.A. Screening methods to determine antibacterial Extract Journal of Pharmaceutical Research International 2020; 32(21): 96-101. activity of natural products. Braz. J. Microbiol. 2007;38:369–380.

[24] Santos, S., Martins, C., Pereira, C., Silvestre, A., & Rocha, S. M. (2019). Current Challenges and Perspectives for the Use of Aqueous Plant Extracts in the Management of Bacterial Infections: The Case-Study of *Salmonellaenterica* Serovars. *In ternational journal of molecular sciences*, *20*(4), 940. https://doi.org/10.3390/ijms20040940

[25] Prashik Parvekar, Jayant Palaskar, Sandeep Metgud, Rahul Maria & Smita Dutta (2020) The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against *Staphylococcus aureus*, Biomaterial Investigations in Dentistry, 7:1, 105-109, DOI: 10.1080/26415275.2020.1796674

[26] Bajpai, Vivek K. & Majumder, Rajib & Park, Jae. (2016). Isolation and purification of plant secondary metabolites using column-chromatographic technique. Bangladesh Journal of Pharmacology. 11. 844. 10.3329

[27] Sharma K and Dabahadker K (2020). Antimicrobial activity and phytochemical screening of *Hyptis suaveolens*. Int J Pharm Sci & Res 2020; 11(1): 438-44. doi: 10.13040/IJPSR.0975-8232.11(1).438-44.

[28] Jithu U Krishnan, Meera George, Ajesh G. Phytochemical status, antibacterial activity and antioxidant property of *Datura metel* L.. Int J Curr Pharm Res 2017;9(3):106-110.

[29] Raji, P., Samrot, A.V., Keerthana, D. *et al*. Antibacterial Activity of Alkaloids, Flavonoids, Saponins and Tannins Mediated Green Synthesised Silver Nanoparticles Against *Pseudomonas aeruginosa* and *Bacillus subtilis*. J Clust

Sci 30, 881–895 (2019). https://doi.org/10.1007/s10876-019-01547-2

[30] Konaté K, Mavoungou JF, Lepengué AN, Aworet-Samseny RR, Hilou A, Souza A, Dicko MH, M'Batchi B. Antibacterial activity against β-lactamase producing methicillin and ampicillin-resistants *Staphylococcus aureus*: fractional inhibitory concentration index (FICI) determination. Ann Clin Microbiol Antimicrob. 2012;11(1):18.