

Antibacterial Activity and Time-kill Assay of *Terminalia catappa* L. and *Nigella sativa* L. against Selected Human Pathogenic Bacteria

Sowmya¹  and Koteswar Anandrao Raveesha^{1,2*} 

¹Center for Innovative Studies in Herbal Drug Technology, Department of Studies in Botany, University of Mysore, Mysuru - 570 006, Karnataka, India.

²Department of Water and Health, Faculty of Life Sciences, JSS Academy of Higher Education and Research Sri Shivaratreeshwara Nagara, Mysuru - 570 015, Karnataka, India.

Abstract

The current investigation aims to test the susceptibility of human pathogenic clinical isolates and MTCC strains to leaf and seed extracts of *Terminalia catappa* and *Nigella sativa*. Disc diffusion assay, micro dilution assay and minimum Bactericidal Concentration investigated the susceptibility of bacteria to the test extracts. The active extract was subjected to phytochemical screening, separation of the phytochemicals by Thin Layer Chromatography, bioactivity guided assay and Time-kill assay. Acetone and methanol extracts of *T.catappa* revealed, significant inhibition of clinical origin *Staphylococcus aureus* followed by *Proteus vulgaris* and the MTCC strains *Staphylococcus aureus*, *Salmonella typhi*, *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Nigella sativa* inhibited the growth of clinical origin *Staph.aureus* and MTCC strain of *Staph.aureus*, *Salmonella typhi* and *B.subtilis*. Minimum inhibitory concentration for all the test bacteria was reported in the range of 5000µg/ml to 9 µg/ml in *T. catappa* extract. Most sensitive being the clinical isolate *Staph. aureus* and *Proteus vulgaris*. The bactericidal concentration for the test bacteria was found to be between 5000µg/ml and 625µg/ml. Phyto-chemical analysis of leaf extracts of *T. catappa* found to have dominated by polyphenols (Terpenoids, steroids, flavonoids, flavones, saponins and tannins) and *N.sativa* extracts recorded the presence of alkaloids, proteins and oils and fats. TLC profiling of the acetone extract revealed many antibacterial active bands. Bands having Retention factor 0.47 and 0.52 were active against the test bacteria. Time kill assay of the acetone extract of *T. catappa* were carried out for the first time. The extract exhibited dose dependent bactericidal and bacteriostatic activity against the clinical isolates.

Keywords: Combretaceae, Terpenoids, Polyphenols, Thymoquinone, Bioautography, Time-kill assay

*Correspondence: karaveesha@gmail.com

(Received: August 31, 2020; accepted: January 26, 2021)

Citation: Sowmya, Raveesha KA. Antibacterial Activity and Time-kill Assay of *Terminalia catappa* L. and *Nigella sativa* L. against Selected Human Pathogenic Bacteria. *J Pure Appl Microbiol.* 2021;15(1):285-299. doi: 10.22207/JPAM.15.1.22

© The Author(s) 2021. **Open Access.** This article is distributed under the terms of the [Creative Commons Attribution 4.0 International License](https://creativecommons.org/licenses/by/4.0/) which permits unrestricted use, sharing, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

INTRODUCTION

Recently there has been increase in the spread of untreatable microbial infections and bacteria cause 90% of the infections¹. World Health Organization reports that infectious diseases cause 50% deaths. The non-selective use of synthetically originated antibiotics, microorganisms have led to the development of multidrug resistance, which poses a serious health concern². The resistance to drug may be caused mostly by the unregulated use of antibiotics and poor hygienic conditions and affects severely in every aspect of life³. The escalating resistance in microorganisms is due to the phenomenon of genetic mutations or acquired antibiotic resistant genes influenced by ill-suited use of antibiotics¹. Additionally antibiotics are associated with unfavorable consequences on the host including hypersensitivity, allergic reactions and immune-suppression⁴. The multiple drug resistance has enforced researchers in search of new drugs with antimicrobial property from different sources like medicinal plants, which serve as acceptable source of novel antimicrobial agents⁵. Rational localization of biologically active components from folk medicines and systemic evaluation will result in finding of novel efficacious drugs, which are potentially active against pathogens⁶. Antimicrobial drugs with efficacious mode of action should be developed in order to surpass the downside of current antimicrobial drugs⁷. A large number of medicinal plants are reported to exhibit antimicrobial activity². Reports of WHO states that medicinal plants would be the best root to acquire diverse of drugs¹. In this context, plants promise a source of natural antimicrobial agents⁸. Antimicrobial activities of plants are ascribed to the presence of phytochemicals like tannins, phenols, alkaloids, terpenoids and flavonoids⁹. The application of plant based drugs in the treatment against pathogens is obtaining great acceptance because of scientific interest and non toxicity properties¹⁰.

Approximately 119 drugs isolated from plants are used in treating infectious diseases worldwide¹¹. Not less than 50% of the drugs which are used in treating clinically infectious diseases accounts to have originated from natural and natural product derivatives¹². Since phytochemicals have the ability to inhibit the

growth of infectious microbes, many plants are still being used for their antimicrobial properties and many other biological activities. In spite of existence of vigorous antimicrobial drugs, the emergence of resistance microbes has created an immense interest in the exploration and the outcome of efficacious drugs¹³.

Terminalia catappa is a large tropical tree of the family Combretaceae¹. Different parts of the plant are used in folklore medicine and studies have revealed various medicinal properties¹⁵. Various research disclose the medicinal uses like microbial inhibition of leaf aqueous and methanolic extract of the plant¹⁶. Bacterial inhibitory activity of leaf aqueous extract¹⁷, anti-inflammatory property of leaf ethanolic extract¹⁸, wound healing activity of bark, antioxidant and radical scavenging activity of leaf aqueous extract, anticancer and anti-aging activity of ethanol and aqueous extract of leaves have also been reported¹⁹. Anti-methicillin potential of phytochemicals of *T.catappa* have also been evident²⁰. Insilico studies of compounds from *T. catappa* are responsible for hepatoprotective and hepatotoxic properties²¹. Extracts of the leaves of the plant also possess anticancer, anti-HIV reverse transcriptase, hepatoprotective, Anti-inflammatory, Anti-hepatitis and Aphrodisiac effects²². Phytochemicals include tannins (Punicalagin, Punicalin, Chebulagic acid, geranin, granitin B), flavonoids (Vitexin, Rutin, Isovitexin), and Terpenoids (Ursolic acid, Asiatic acid) which are responsible for therapeutic activity²³.

Nigella sativa, belongs to the family Ranunculaceae²⁴. It is native to Mediterranean regions such as South west Asia, Southern Europe and North Africa²⁵. Seeds contain yellowish volatile oil, fixed oil, alkaloids, saponins, minerals and vitamins²⁶. The reported studies related to *Nigella sativa* have illustrated wide range of biological activities such as antioxidant²⁷, anti-inflammatory²⁸, antidiabetic²⁹, anti prostate cancer effects³⁰, antibacterial³¹, immunomodulatory effect³², and hepatoprotective³³.

Secondary metabolite analysis found that seeds contain two types of alkaloids, isoquinone alkaloids (Nigellimine – N oxide) and pyrazole alkaloids (Nigellidine and Nigellidine)³⁴. Essential phytoconstituents also include thymoquinone,

P-cymene, 4- Terpineol, carvacrol, t- anethol, longifoline, thymol, thymohydroquinone and dithymoquinone additionally they have a water soluble pentacyclic triterpene, alpha- hedarin and saponins as anticancer agents³⁵.

MATERIALS AND METHODS

Preparation of the plant extract

Mature leaf material was collected from fields in and around Mysuru, Karnataka. The leaves were shade dried and powdered using laboratory blender. *N. sativa* seeds were collected from the local market, powdered and used for successive solvent extraction by soxhlet extractor using polar and non-polar solvents. After extraction the solvents were evaporated to dryness under reduced pressure and preserved the extracts at 4°C for future analysis.

Microorganisms and growth conditions:

Human pathogenic bacteria were obtained from MTCC Chandigarh, Viz, *B.subtilis* (MTCC 121), *B.cereus* (MTCC 1272), *Pseudo.aeruginosa*, (MTCC424) *Salm. typhi* (MTCC 733), *Escherichia coli* (MTCC 7410) and *Staph.aureus* (MTCC 7443). Human pathogenic clinical isolates were provided by Microbiology department, Government Medical College, Mysuru, Viz, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Salmonella typhi*. The pathogens were maintained on Muller Hinton agar in glass tubes. Bacteria were resuscitated by sub culturing from stock onto Muller Hinton agar plates followed by incubation overnight for their optimum growth.

Antibacterial activity of the plant extracts

Antibacterial susceptibility test by Disc diffusion assay

Antibacterial potency of the test plant extracts were done by disc diffusion method employing the CLSI M02-A document³⁶. Inoculums were prepared and adjusted to McFarland standard with cell density of 1 to 1-2 X10⁸ CFU/ml. Standardized inoculum (0.1ml) was swabbed to the previously solidified MH agar plate. Sterile discs were loaded with test plant extract (100mg/ml) and impregnated onto the plates followed by incubation for 18-24 hours at 35 ±2°C. Standard antibiotics and solvents were employed as positive and negative control. All experiments were carried out in triplicates. Zone of inhibition (ZOI) around the disc was measured in mm.

Microbroth dilution method for determining Minimum inhibitory concentration (MIC) and Minimum Bactericidal Concentration (MBC)

MIC was established by employing 96 well microtiter plate according to the CLSI M07-A9 document³⁷. The test plant extract, which showed the inhibitory activity, were selected for determining MIC and MBC. The test plant extract were serially diluted two fold to obtain concentration of 5000-9µg/ml. The experimental set up included reference drug controls and solvent as positive and negative controls respectively. Aliquot of standardized inoculum (1.5 X 10⁸ colony forming units/ml) was added to all the wells. The micro dilution plate was covered to avoid drying followed by incubation. Inhibition of bacterial growth was confirmed by addition of 20µl of aqueous solution of 2, 3,5-Triphenyl tetrazolium chloride (TTC) and re-incubated for 4-5h. The colorless well was designated as the MIC, which inhibited the growth of bacteria. Change of colour from colourless to pink indicated the presence of viable cells. MBC was ascertained by sub culturing 10µl of test dilution from the lowest concentration well by streaking on the previously sterilized and solidified MH agar with overnight incubation at optimum temperature. The well, which gave no bacterial colony on the agar medium, was designated as MBC.

Phytochemical composition of the active test extracts

The test extracts were subjected to qualitative phytochemical analysis according to the established procedures to determine the presence of different class of secondary phytochemicals^{38,39}.

Thin layer chromatography (TLC) of the active extracts

The active extracts were subjected for separation of phytochemicals. Based on the review of literature and slight modification, EMW (Ethyl acetate: methanol: water) with 40:5.4:4 ratio showed better separation of the compounds and it was best-suited eluent system for the separation of phytochemicals from members of Combretaceae. The retention factor (Rf) of the separated phytochemicals was calculated⁴⁰.

TLC bioautography

Agar overlay technique⁴¹ was adopted with minor modifications to locate the antibacterial bands. The TLC plates (silica gel G f₂₅₄, Merk) were

spotted with test plant extract (acetone extract) and developed in the pre saturated developing chamber using EMW as eluent system. After the solvent front reached the optimum distance the chromatogram was removed and visualized under long and short UV to locate the separated compounds. The chromatogram was dried overnight and subjected to overlay bioautography.

One milliliter of standardized test inoculum was mixed with 10ml molten Muller Hinton agar. TLC chromatograms were placed in the sterilized plates and thin layer of media inoculated with the test bacteria was flooded on the chromatogram. Plates were incubated overnight at 37°C. ZOI around the separated phytochemicals can be seen as a clear area without the growth of the test bacteria. As

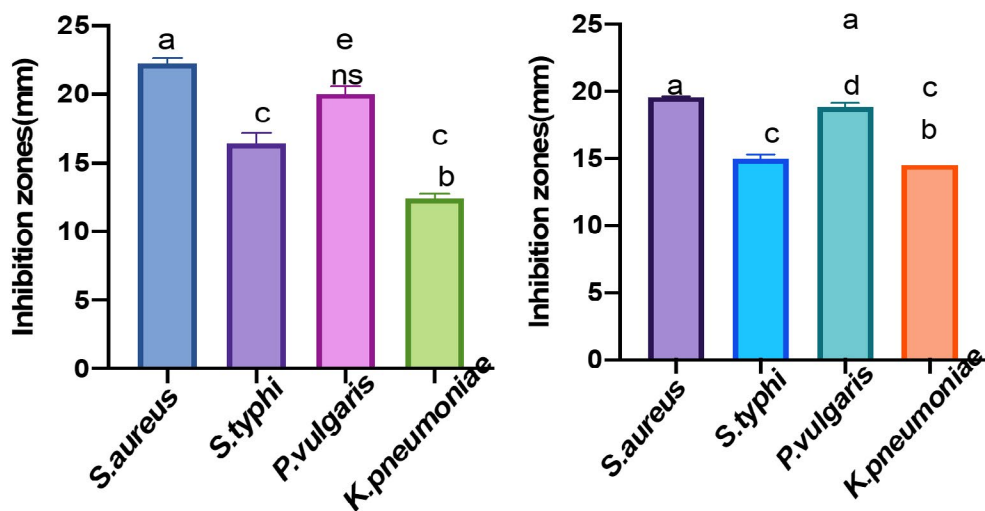


Fig. 1 & 2. Antibacterial activity of Acetone extract (Fig:1) and Methanol extract (Fig :2) of *T. catappa* on human pathogenic clinical isolates. Data are represented in mean \pm SEM. Different letters in the bars depict the significant difference at $P < 0.05$. Error bars signify standard error. Inhibition zones are presented in millimeter (mm).

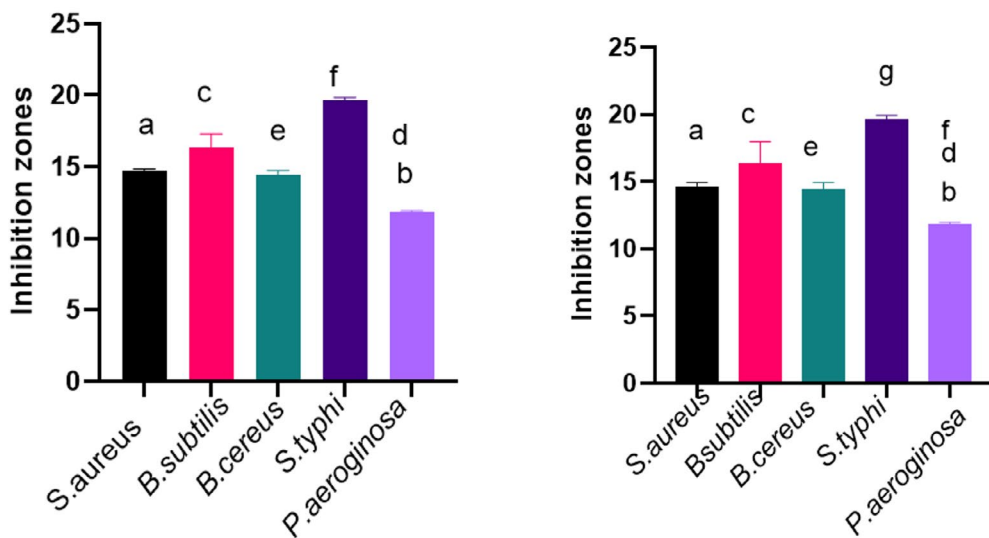


Fig. 3 & 4. Antibacterial activity of Methanol extract (Fig:3) and Acetone extracts (Fig :4) of *T. catappa* on human pathogenic bacteria (MTCC). Data are represented in mean \pm SEM. Different letters in the bars indicate statistical difference ($P < 0.05$). Error bars signify standard error. Inhibition zones are presented in millimeter (mm).

a confirmatory test the chromatograms were flooded with microbiological agar containing TTC and further incubates for 30 minutes for optimum colour development. The ZOI is seen as white zone against the pink background.

Time kill assay

Protocol of kill time assay was adopted⁴² with slight modifications. The active extract of *T. catappa* was set at a concentration of 4MIC and 8MIC. A control tube was maintained without the test extracts. Each tube was then inoculated with standardized suspension of clinical isolates of *S.aureus* and *P.vulgaris* At prefixed time points aliquots were withdrawn from each concentration diluted serially (10^{-1} to 10^{-4}) and aliquots were plated on agar plates. Colony counts were performed after overnight incubation at 37°C in ambient air. Colony counts were averaged and expressed as \log_{10} cfu/ml.

Satattitcal analysis

Values are presented as Mean \pm SEM. ANOVA was employed to determine the difference in specific means followed by tukey’s post hoc test at $P<0.05$. Graphpad prism (version 8) and Microsoft excel 2007 was used in generating graphs and analyzing the data.

RESULTS

Disc diffusion assay

The test organisms exhibited different sensitivity for the extracts tested which is shown in Fig. 1 to 4. All the test pathogens exhibited significant sensitivity towards acetone and methanol extracts of *T.catappa*. Acetone extract had good inhibitory activity against the test organisms in contrast with the methanol extract. Significant inhibition was observed against the clinical isolate *Staph.aureus* (22.5mm) followed

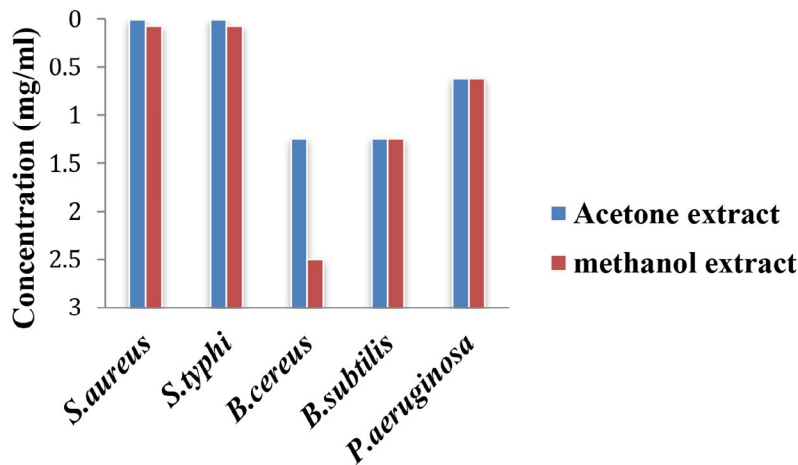


Fig. 5. MIC of active extracts of *T. catappa* on human pathogenic bacteria.

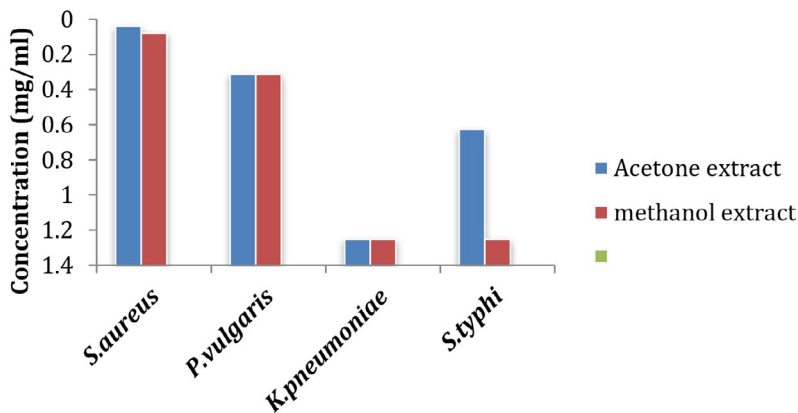


Fig. 6. MIC of active extracts of *T. catappa* on human pathogenic clinical isolates.

by *P.vulgaris* (20.5mm), *Salm.typhi* and *Kleb.pneumoniae* with inhibition zone 16.4mm and 12.4mm respectively. The MTCC strains also exhibited sensitivity towards the acetone and methanol extracts with zone of inhibition ranging between 11.7mm and 23.3mm. Hexane extract of *N.sativa* inhibited the growth of clinical isolate of *Staph.aureus* with inhibition zone 19.08mm,

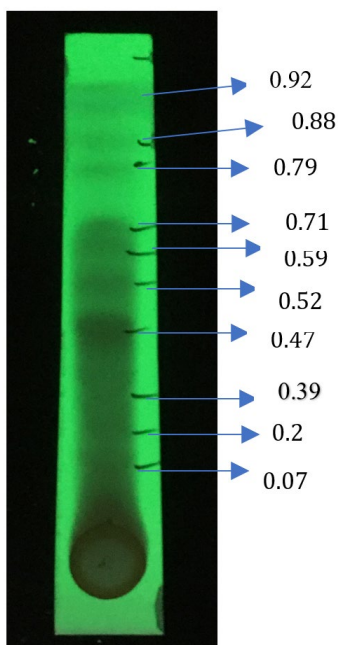


Fig. 7. Chromatogram of acetone extract of *T.catappa* leaf eluted with Ethyl acetate, Methanol and Water solvent system (40:5.4:4) showing clear separation of phytocompounds.

Table 1. Minimum Inhibitory and Minimum Bactericidal Concentration and of acetone and methanol extract of *T.catappa* on human pathogenic bacteria (MTCC)

MTCC strains	Acetone extract		Methanol extract	
	MIC	MBC	MIC	MBC
<i>S.aureus</i>	9	*	78	*
<i>S.typhi</i>	9	625	78	5000
<i>B.cereus</i>	1250	2500	2500	5000
<i>B.subtilis</i>	1250	*	1250	*
<i>Paeruginosa</i>	625	5000	625	5000

FN: Values presented in µg/ml; *: Indicates no MBC established

Staph.aureus (MTCC 7443) and *Salm.typhi* (MTCC 733) with zone inhibition 11.25mm and 15.9mm respectively.

Micro dilution assay

Considering the results obtained from disc diffusion assay, deducing the lowest inhibitory concentration (MIC) of active extracts was considered necessary and the results are shown in Fig. 5 and 6.

Of all the organisms tested, the clinical isolate *Staph.aureus* and *P. vulgaris* was the

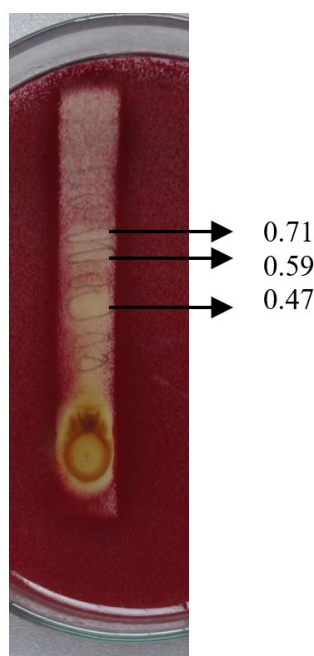


Fig. 8. Agar overlay bioautography of acetone extract of *T.catappa* leaf showing inhibition of *P. vulgaris*(Clinical isolate) at Rf values 0.71. 0.59 and 0.47.

Table 2. Minimum Inhibitory and Minimum Bactericidal Concentration of acetone and methanol extract of *T.catappa* on human pathogenic clinical isolates

Clinical isolates	Acetone extract		Methanol extract	
	MIC	MBC	MIC	MBC
<i>S.aureus</i>	39	1250	78	5000
<i>P.vulgaris</i>	312	5000	312	5000
<i>K.pneumoniae</i>	1250	5000	1250	5000
<i>S.typhi</i>	625	5000	1250	5000

FN: Values presented in µg/ml

most sensitive to the acetone extract with lowest concentration of 39µg/ml and 312µg/ml respectively, *Kleb.pneumoniae* and *Salm.typhi* were less susceptible with concentration 1250µg/ml and 625µg/ml respectively. The acetone extract also exhibited inhibitory effect at the lowest concentration against all the MTCC strains tested except *E.coli* with concentrations between 1250µg/ml and 9µg/ml.

Methanol extract showed inhibitory activity towards the test pathogens but slightly less effective compared to the acetone extract having concentration between 5000µg/ml and 625µg/ml for clinical isolated bacteria. The minimum concentration of methanol extracts required against *B.subtilis* (MTCC 121) *Salm.typhi* (MTCC 733), *Staph.aureus* (MTCC 7443), *Pseudo.aeruginosa* (MTCC 424), and *B. cereus* (MTCC 1272) was in the range between 1250µg/ml and 78µg/ml.

Minimum Bactericidal Concentration

Acetone extract reported bactericidal effect at 1250µg/ml for clinical isolate *Staph.aureus* where as the bactericidal effect for *Kleb.*

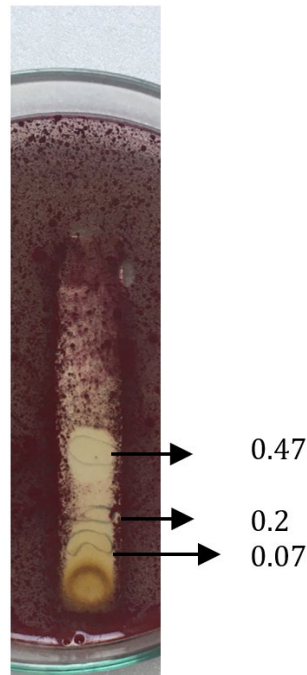


Fig. 9. Agar overlay bioautography of acetone extract of *T.catappa* leaf showing inhibition of *S.aureus* (MTCC 7443) at Rf values 0.47, 0.2 and 0.07.

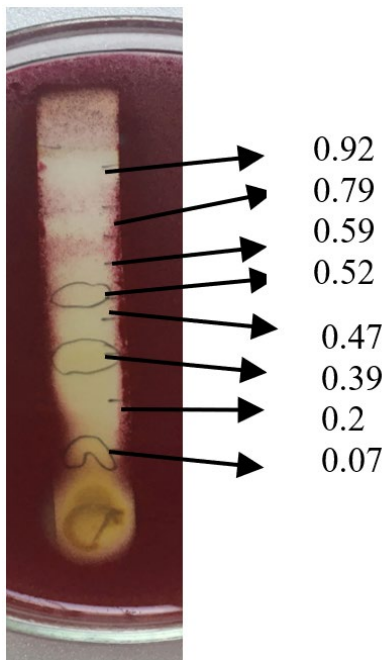


Fig. 10. Agar overlay bioautography of acetone extract of *T.catappa* leaf showing inhibition of *S.typhi* (MTCC 733) at Rf values 0.92, 0.79, 0.59, 0.52, 0.47, 0.39, 0.2 and 0.07.

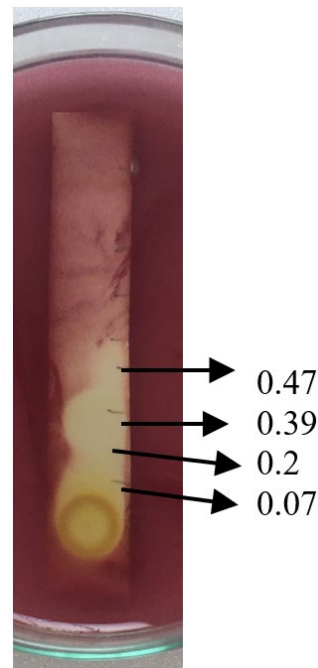


Fig. 11. Agar overlay bioautography of acetone extract of *T.catappa* leaf showing inhibition of *S.aureus* (clinical isolate) at Rf values 0.47, 0.39, 0.2 and 0.07.

pneumoniae, *Salm.typhi* and *P. vulgaris* was 5000µg/ml. MBC against *Salm.typhi* (MTCC 733), *B.cereus* (MTCC 1272) and *Pseudo.aeruginosa* (MTCC 424) was at the concentration of 625 µg/ml, 2500 µg/ml and 5000 µg/ml respectively. On the contrast bactericidal action of methanol extract was 5000µg/ml for all the bacterial clinical isolates and the MTCC strains except *Staph.aureus* (MTCC 7443) and *B.subtilis* (MTCC 121), which did not show any bactericidal activity at the tested concentration.

Results of phytochemical analysis presented in Table 3. Broad group of phytochemicals were present in the acetone and methanol extract like terpenoids, flavonoids, saponins, cardiac glycosides, phenols and tannins. Hexane extract of *Nigella sativa* was dominated by alkaloids, oils and fats.

TLC profiling and bioautography

Thin layer chromatography of acetone extract using EMW solvent system was capable of eluting bands with Rf values 0.07, 0.20, 0.39, 0.47, 0.52, 0.59, 0.71, 0.79, 0.88 and 0.92.

Agar overlay bioautography of acetone extract of *T. catappa* showed clear zone of inhibition at Rf values 0.47, 0.2 and 0.07 against clinical isolate *S. aureus* (Fig. 11) and *Proteus vulgaris* (Fig. 8) was inhibited by the bands with Rf values 0.71, 0.59 and 0.47. *S.typhi* (MTCC 733) (Fig. 10) was also inhibited by the bands with Rf value

0.92, 0.79, 0.59, 0.52, 0.47, 0.39, 0.2 and 0.07. Bands with Rf value of 0.47, 0.2 and 0.07 strongly inhibited the *Staph.aureus* (MTCC 7443) (Fig. 9). Clear zone of inhibition around the separated bands was indicative of antibacterial property of the compounds present.

Colorless areas indicate inhibition of the test organisms by the separated phytochemicals.

Kill time assay

The time kill profile for clinical isolates *Staph. aureus* and *P.vulgaris* are presented in Table 4 showing the reduction in viable cell counts at particular time intervals. For both bacterial strains a separate time kill profile was produced over a period of 24 hours of incubation following inoculation. Difference in the viable counts for both bacterial strains were more after 4h post inoculation. At concentration corresponding to 8MIC bactericidal activity as observed at time point of 2-4h for *Staph.aureus* (Fig. 13) after which regrowth of the bacteria was observed indicating concentration dependent killing of the test bacteria. However, 4MIC concentration did not kill the bacteria but slowed down the growth compared to initial control. For *P. vulgaris* (Fig. 12) maximum reduction of viable cell counts was seen after 24h of incubation at 8 MIC concentration with near bactericidal effect of 3.37 log₁₀ Cfu/ml and 4MIC concentration reduced the viable cell counts after 8h of incubation with 3.9log₁₀

Table 3. Qualitative phytochemical analysis of the test plants

Secondary metabolites	Tests	<i>T.catappa</i>		<i>N.sativa</i>
		Acetone	Methanol	N-hexane
Terpenoids	Salkowski test	+	+	-
	Liebermann test	+	+	-
Alkaloids	Mayers test	-	-	+
	Wagners test	-	-	+
Flavonoids	Lead acetate test	+	+	-
	Shinoda test	+	+	-
	Alkaline test	+	+	-
Saponins	Froth test	+	+	-
Tannins	Gelatin test	+	+	-
Oils and fats	Oils and fats	-	-	+
Phenols	Fecl3 test	+	+	-
Cardiac glycosides	Killer-kellani test	+	+	-

FN: +: Present; -: Absent

Table 4. Time kill profile of acetone extract of Terminalia catappa showing reduction of viable cell counts at different time intervals.

Time intervals	0hr	2hr	4hr	6hr	8hr	24hr	0hr	2hr	4hr	6hr	8hr	24hr
Test bacteria	<i>S.aureus</i> (Log ₁₀ cfu/ml)						<i>P.vulgaris</i> (Log ₁₀ cfu/ml)					
Concentration												
Control	2.89	3.19	3.32	4.85	5.87	6.19	3.5	4.38	4.91	5.78	5.8	6.07
4MIC	2.82	3.20	2.1	3.38	3.74	6.24	3.21	4.2	4.02	4.07	3.9	5.76
8MIC	2.38	3.0	3.0	3.25	3.27	6.11	3.40	3.4	3.74	3.68	3.84	3.37

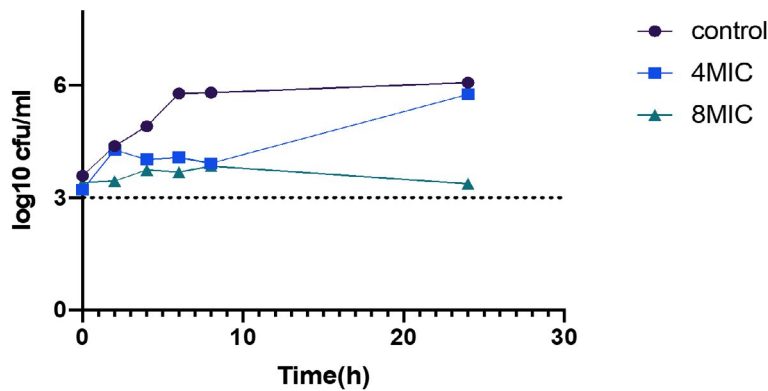


Fig. 12. Time kill curve of Acetone extract on *P. vulgaris* (Clinical isolate).

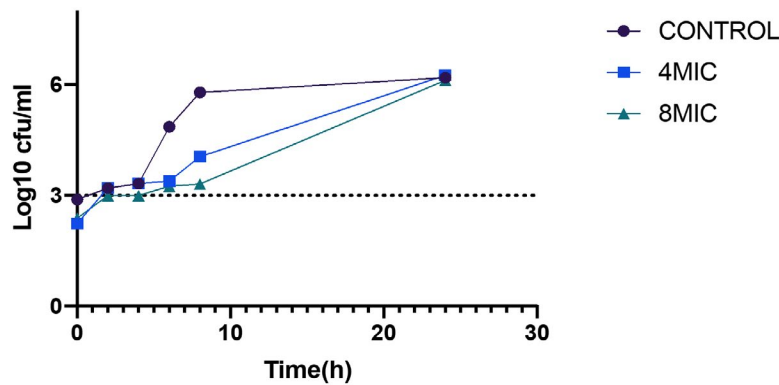


Fig. 13. Time kill curve of Acetone extract on *Staph.aureus* (Clinical isolate).

Cfu/ml exhibiting the bacteriostatic action of the extract. For both the bacteria tested the viable colonies were reduced post inoculation after which the colonies regrew until the end of time course. However, there was delay in the exponential growing phases of the test bacteria. 8MIC concentration was more effective than 4MIC for both the test bacteria indicating the extract is

dose and time dependent killing effect irrespective of the bacteria.

DISCUSSION

The present investigation reports the acetone and methanol extract of *T.catappa* leaf and hexane extract of *N.sativa* seeds demonstrated the antibacterial activity against the human

pathogenic bacteria and clinical isolates. The preliminary method for evaluating the sensitivity of the test bacteria towards the plant extract is inhibition zone testing by disc and well diffusion assay. However, to test the efficacy of the extracts, broth dilution assays are also used preferably microbroth dilution assay⁴¹.

Acetone and methanol extract of *T.catappa* demonstrated varying level of activity with inhibition zones ranging between 11.66 and 23.33 mm for all the test bacteria.

The results are in accordance with the previous reports where in polar solvent (methanol and aqueous) extracts of *T. catappa* leaves in addition to twenty other plants were evaluated against bacteria (Gram positive and gram negative) and found that majority of the test strains were susceptible to methanol extract having inhibition zone 5-18mm⁴⁶. The differences in the antibacterial activity can be due to the different chemical composition and the distinct mechanism of action of their bioactive constituents⁴³. The results can also be attributed with previous reports, which recorded the aqueous extracts of *T.catappa* exhibited inhibitory activity against *Staph. aureus*, *Kleb.pneumoniae*, *E.coli* and *Candida albicans*⁴⁴. Several other reports have revealed the antimicrobial activity of leaves and fruits of *T.catappa* on different gram positive and negative bacteria⁴⁵. The susceptibility results obtained by disc diffusion assay of leaf methanol extract of *T. catappa* against *Staph.aureus*, *pseudo. aeruginosa*, *B.subtilis* and a clinical isolate *Staph.aureus* demonstrated that gram-positive organisms were more susceptible⁴⁶. Polyphenols are reported to have antibacterial activity⁴⁷. Various polyphenols isolated from *T.catappa* like catappanin, geranin, gallantonic, leutolin, apigenin, orientin, isovitexin, catachien, kampferol, genistein, quercetin, ellagic acid, chlorogenic acid, ferulic acid, arjunetin,, ursolic acid, gallic acid, arjunolic acid, betulinic acid and several compounds from *T.catappa* have been reported. The above isolated compounds from the plant could be accredited to the invitro susceptibility of bacteria found in another study⁴⁸. Several other workers⁴⁹⁻⁵¹ have also reported the antibacterial properties of various other parts of *T. catappa* against human pathogenic bacteria. Our results exhibit a wide spectrum inhibitory activity towards both gram-positive and negative

bacteria. We report strong antibacterial inhibition by polar solvent extracts (acetone and methanol) of *T.catappa* against clinical isolate *Staph.aureus* followed by *Salm.typhi* (MTCC 733) and clinical isolate *P.vulgaris*.

The hexane extract of *N.sativa* exhibited inhibitory activity against clinical isolate *Staph. aureus*, *Salm. typhi* (MTCC 733), *Staph. aureus* (MTCC 7443) and *B. subtilis* (MTCC 121). The hexane extract of *N.sativa* showed susceptibility against gram positive and negative bacteria indicating wide range inhibitory activity. The results are in agreement with other workers where methanol and N-hexane extract of *N. sativa* showed considerable good activity against the test pathogens showing varying zone of inhibition with varying dilutions. Gram-positive *S.epidermidis* was most sensitive to the extracts⁵². Aqueous and alcoholic extract of seeds of *N.sativa* against bacteria and fungi was reported and all the extracts posed different efficacy against the test organisms⁵³.

Evaluation of essential oil for antibacterial activity, which showed the suppression of test pathogens exhibiting varying zone of inhibition⁵⁴. The results can be related to other reports where the essential oil and oleoresins exhibited the significant inhibition of test organisms⁵⁵. N-hexane extract and seed oil of *N.sativa* on selected human pathogenic bacteria was evaluated and found that the test microbes were inhibited by both extract and oil⁵⁶. The results obtained in the study are in concordance with the previous studies. The methanol extracts obtained from the seeds were evaluated for antibacterial activity by and found that *Staph. aureus* and *Pseudo.aeruginosa* were sensitive to the extracts⁵⁷. N-hexane, methanol and aqueous seed extract of *N.sativa* was evaluated for their invitro susceptibility activity where methanol extract showed considerable inhibition of *Staph. aureus*, *Escherichia coli* and *Salm. enterica*⁵⁸. The efficacy of *N.sativa* oil is attributed to its quinone constitutes in the fixed and essential oil, which is, endowed with thymoquinone a significant bioactive constituent making up 30-48% of total constituents. Other functional constituents include p-cymene, carvacrol, thymohydroquinone, dihydrothymoquinone, thymol, α -thujene, t-anthole, β -pinene, α pinene and γ -terpinene⁵⁹.

The MIC values of *T.catappa* showed, that

acetone extract was more potent in inhibiting the test bacteria and methanol extract was slightly less potent than the acetone extract. However, hexane and chloroform extract had no activity against the test strains owing to the fact that polar component of the extract is posing the antibacterial action. The acetone extract showed strong inhibition of clinical isolate *Staph. aureus* (MIC; 39µg/mL) and *P. vulgaris* (MIC; 625µg/mL). MIC values of acetone extract for MTCC bacteria ranged between 9µg/ml and 1250µg/ml. Methanol extract posed the lowest inhibitory concentration (MIC) from 78µg/ml to 1250µg/ml for both clinical isolates and MTCC strains. However, the methanol extract exhibited moderate inhibitory activity compared to acetone extract. MBC values were more than the MIC. When higher concentration used there might result in bactericidal effect. Some researchers have reported the antimicrobial agents having MIC values between 1.60mg/ml to 8mg/ml as frail microbial inhibitors⁶⁰. None of the extracts showed MIC values greater than 8mg/ml. In agreement with our study the previous reports where the crude extracts of leaves of *T. catappa* and eight other plants were evaluated for MIC against human pathogenic bacteria and reported that pathogens were inhibited at the concentration from 16mg/ml to 0.015mg/ml⁶¹. The MIC of leaves of *T.catappa* was evaluated at concentration ranging from 100mg/ml to 350mg/ml against a panel of bacteria. Bacteria included *Staph. aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Salmonella typhi* and *Shigella dysenteriae*⁶². Comparative evaluation of leaf aqueous and methanol extracts of *Combretum* and *Terminalia* spp. of southern Africa were evaluated, which reported that both the species had broad spectrum antimicrobial activity inhibiting both bacteria and fungi. The MIC values inhibiting the test strains were less than 1000 µg/ml where in *Bacillus subtilis* had least MIC ranging from 124-578µg/ml *Staphylococcus aureus* (395-770 µg/ml), *K.pneumoniae* (318-531µg/ml) *P.aeruginosa* (36-512µg/ml)⁶³. Our work reports that the test strains were inhibited at the lowest concentration with special reference to clinical isolate of *Staph. aureus* and *Proteus vulgaris*. Members of the *Terminalia* genus have a long history in traditional system of medicines and are used in

several continents for treating numerous diseases including cardiovascular effects, wound healing, abdominal disorders, conjunctivitis, hypertension, pneumonia, gastric ulcers, jaundice, leprosy, edema and skin diseases⁶⁴. Various species of *Terminalia* have been utilized in treating infections caused microbes and many current researchers have documented their antibacterial potential. Indian and southern Asian *Terminalia* species have been particularly studied well. Antibacterial susceptibility of wide panel of organisms has been specifically documented in *Terminalia arjuna*, *Terminalia bellirica* *Terminalia catappa* and *Terminalia chebula*⁶⁵.

Qualitative secondary metabolite screening of active extracts (acetone and methanol) exhibited the dominance of terpenoids, phytosterols, flavonoids, flavones and saponins. These compounds have been previously reported by earlier workers⁶⁶⁻⁶⁹. The leaves of *Terminalia catappa* contain chebulagic acid corilagin, gentisic acid, geranin, granatin B, kaempferol, punicalagin, punicalin, quercetin, terfavin A and terflavin B⁷⁰. The study is in agreement with the ⁷¹ wherein quercetin was identified in the leaves of *Terminalia catappa* along with other components like flavonoids, carotenoids and other phenolic compounds, which is responsible for the traditional use of the plant.

Hexane extract of *N. sativa* revealed the presence of alkaloid, which is the major component of the seeds. The results are in accordance with the earlier work⁷², which reported the occurrence of tannins, alkaloids, flavonoids and sterols.

TLC bioautography helps in locating the antibacterial active spots on the chromatogram. EMW solvent system was capable in eluting the antibacterial compound in acetone extract of with Rf value of 0.59, 0.52, 0.47 and 0.39. Bioautography studies revealed that most of the separated compounds had antibacterial potential, which can be due to the occurrence of polyphenols in the test plant extract, which is also confirmed from the qualitative phytochemical analysis. Time kill studies are important because they provide information about pharmacodynamics of the antibacterial agent⁷³. Extracts showed variable kinetics against test bacteria. Time kill findings displayed different levels of time dependent

and concentration dependent inhibition of the organisms. Bacteriostatic and bactericidal activity was displayed by the extract mainly against clinical isolates but regrowth of the test bacteria occurred after specific time interval which could be attributed to the use of lesser concentration of the extracts and also the gram positive and negative bacteria differ in cell wall and the membrane composition which regulate their susceptibilities to plant metabolites. At higher concentration bactericidal activity may be achieved invitro. Time kill kinetics of *T.catappa* against selected human pathogenic bacteria was carried out for the first time, however there are fewer reports related to time kill kinetics of fungi⁷⁴ but, reports on bacteria are scarce to the best of our knowledge.

CONCLUSION

The results achieved in this study indicate that *T.catappa* and *N.sativa* are potential sources of antibacterial agents against the bacterial clinical isolates in particular. The acetone extract of *T.catappa* exhibited wide spectrum activity against (gram-positive and gram-negative) MTCC and clinical isolates. The extracts also inhibited the test bacteria at a very minimal concentration. Bioautography profile of acetone extract resulted in potent antibacterial bands, which exhibited inhibition both MTCC and clinical isolates was observed. Time kill assays indicated time and concentration dependent activity. Both bacteriostatic and bactericidal activity was observed against clinical isolates. Use of higher concentrations could lead to bactericidal activity against the test bacteria. The extracts can be employed in improving human health in the prevention of treatment of infectious diseases and to fight the emergence and spread of resistant organisms. Further pure compound isolation, structure elucidation and mechanism of action of the isolated molecule followed by invivo toxicity studies may lead to potential drug in the ongoing search for antimicrobial botanicals.

ACKNOWLEDGMENTS

Authors are thankful to Center for Innovative Studies in Herbal Drug Technology, Department Of Studies in Botany, University of Mysore, Manasagangotri, Mysuru, Karnataka for

providing the facilities and to the Department of Microbiology, Mysore Medical College and Research Center for providing the clinical bacterial isolates.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors have made substantial, direct and intellectual contribution to the work and approved it for publication.

FUNDING

Authors are thankful to ICMR-New Delhi and VGST-CISEE, Government of Karnataka for financial assistance. funding grant number No.45/19/2018/TM/BMS

DATA AVAILABILITY

All datasets analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

REFERENCES

1. Mozirandi W, Mukanganyama S. Antibacterial activity and mode of action of *Veronia adoensis* (Asteraceae) extracts against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Journal of Biologically Active Products form Nature*. 2017;7(5):341-357. doi: 10.1080/22311866.2017.1378922
2. Tang QL, Kang AR, Lu C X. Phytochemical analysis , antibacterial activity and mode of action of methanolic extract of *Scutellaria barbata* against various clinically important bacterial pathogens. *Intl J Pharmacol. (IJP)*. 2016;12(2):116-125. doi: 10.3923/ijp.2016.116.125
3. Nijmoh DL, Assob JCN , Mokake SE Nyhalah DJ Yinda CK, Sandjon B. Antimicrobial activity of a Plethora of medicinal plant extract and hydrolates against human pathogenic and their potential to reverse antibiotic resistance. *Int J Microbiol*. 2015;2015:547156. doi: 10.1155/2015/547156
4. Saranraj P, Sivasakthi S. Medicinal plants and its antimicrobial properties: A review. *Global Journal of Pharmacology*. 2014;8(3):316-327.
5. Karaman I, Sahin F, Gulluce M, Ogutcu H, Sengul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of *Junipers oxycedrus* L. *J Ethnopharmacol*. 2003;85(2-3):231-235. doi: 10.1016/S0378-8741(03)00006-0
6. Ghosh A, Das B K, Roy A Mandal B, Chandra G. Antibacterial activity of some medicinal plant extracts.

- J Nat Med.* 2008;62(2):259-262. doi: 10.1007/s11418-007-0216-x
7. Orhan DD, Ozcelik B, Ozgen S, Ergun F. Antibacterial, Antifungal and Antiviral activities of some flavonoids. *Microbiol Res.* 2010;165(6):496-504. doi: 10.1016/j.micres.2009.09.002
 8. Kusuma IW, Murdiyanto, Arung ET, Syafrizal, Kim YU. Antimicrobial and Antioxidant properties of medicinal plants used by the Bentian tribe from Indonesia. *Food Science and Human Wellness.* 2014;3(3-4):191-196. doi: 10.1016/j.fshw.2014.12.004
 9. Khan R, Islam B, Akram M, et al. Antimicrobial activities of five herbal extracts against multidrug resistant (MDR) strains of bacteria and fungus of clinical origin. *Molecules.* 2009;14(2):580-597. doi: 10.3390/molecules14020586
 10. Turker H, Yildirim B. Screening for antibacterial activity of some Turkish plants against fish pathogens: a possible alternative in the treatment of bacterial infections. *Biotechnol Biotechnol Equip.* 2015;29(2):281-288. doi: 10.1080/13102818.2015.1006445
 11. Mahida Y, Mohan JSS. Screening of Indian plant extracts for antibacterial activity. *Pharm Biol.* 2006;44(8):627-631. doi: 10.1080/13880200600897551
 12. Zengin G, Uysal A, Gunes E, Aktumsek A. Survey of phytochemical compositions and biological effects of three extracts from wild plant (*Cotoneaster nummularia* Fisch. Et Mey.): a potential source for functional food ingredients and drug formulations. *PLoS ONE.* 2014;9(11):e113527. doi: 10.1371/journal.pone.0113527
 13. Gobalakrishnan R, Kulandaivelu M, Bhuvaneshwari R, Kndavel D, Kannan L. Screening of wild plants species for antibacterial activity and phytochemical analysis of *Tragia involucrate* L. *J Pharm Anal.* 2013;3(6):460-465. doi: 10.1016/j.jpaha.2013.07.001
 14. Eddy NO, Ekwumemgbo PA, Mamza PAP. Ethanol extract of *Terminalia catappa* as a green inhibitor for the corrosion of mild steel in H₂SO₄. *Green Chem Lett Rev.* 2009;2(4):223-231. doi: 10.1080/17518250903359941
 15. Venkatalakshmi P, Brinda P. Antimicrobial activities of aqueous extracts of different parts of *Terminalia catappa* L. *Int J Curr Microbiol App. Sci.* 2016;5(12):493-498. doi: 10.20546/ijcmas.2016.512.053
 16. Nair R, Chanda S. Antimicrobial activity of Terminalia catappa, Manilkara zapota and Piper betel leaf extract. *Indian J Pharm Sci.* 2008;70 (3):390-393. doi: 10.4103/0250-474X.43012
 17. Tanganna JC, Quanicco JP, Perono RMG, Armor EC, Rivera WL. Tannin rich fraction from *Terminalia catappa* inhibits quorum sensing (QS) in Chromobacterium violaceum and the QS- controlled biofilm maturation and Las A staphylolytic activity in *Pseudomonas aeruginosa*. *J Ethnopharmacol.* 2011;134(3):865-871. doi: 10.1016/j.jep.2011.01.028
 18. Fan YM, Xu LZ, Gao J, et al. Phytochemical and antiinflammatory studies on *Terminalia catappa*. *Fitoterapia.* 2004;75(3-4):253-260. doi: 10.1016/j.fitote.2003.11.007
 19. Anand AV, Divya N, Kotti PP. Pharmacogn Rev. An updated review of *Terminalia catappa*. *Pharmacogn Rev.* 2015; 9(18): 93-98. doi: 10.4103/0973-7847.162103
 20. Ravi L, Jindam D, Kumaresan S, Selvaraj VV, Reddy J. Anti-methicillin resistant *Staphylococcus aureus* potential of phytochemicals in Terminalia catappa and their proposed insilico mechanism of action. *Asian J Pharm Clin Res.* 2019;12(10):133-137. doi: 10.22159/ajpcr.2019.v12i10.34705
 21. Packiri swamy V, Vijayalakshi K. Molecular docking studies of the compounds form *Pergularia daemia* and *Terminalia catappa* Linn. leaf extracts with CYP2 E1, GST, UDP-Glucuronyl transferase and Nrf2 binding site in KEAP1, IL6. *Int J Pharm Sci.* 2018;9(5):2037-2045.
 22. Divya N, Rengarajan RL, Radhakrishnan R, et al. Phytotherapeutic efficacy of medicinal plant *Terminalia catappa* L. *Saudi J Biol Sci.* 2019;26(5):985-988. doi: 10.1016/j.sjbs.2018.12.010
 23. Minninel FS, Junior CSL, Espanha LG, et al. Characterization and quantification of compounds in the hydroalcoholic extract of leaves for *Terminalia catappa* Linn. (Combretaceae) and their mutagenic activity. *J Evid Based Complementary Altern med.* 2014;2014:1-11. doi: 10.1155/2014/676902
 24. Nadaf NH, Gawade SS, Muniv AS, Waghmare SR, Jadhav DB, Sonawane KD. Exploring antiyeast activity of *Nigella sativa* seed extract. *Ind Crops Prod.* 2015;77:624-630. doi: 10.1016/j.indcrop.2015.09.038
 25. Butt AS, Nisar N, Ghani N, Altaf I, Mughal TA. Isolation of thymoquinone form *Nigella sativa* L. and *Thymus vulgaris* L. and its antiproliferative effect on HeLa cancer cell lines. *Trop J Pharm Res.* 2019;18(1):37-42. doi: 10.4314/tjpr.v18i1.6
 26. Ramadan MF. nutritional value and applications of *Nigella sativa* essential oil. A mini review. *Journal of Essential Oil Research.* 2015;27(4):271-275. doi: 10.1080/10412905.2015.1045564
 27. Zhao J, Xu F, Huang ZY, et al. evaluation on Anti inflammatory, Analgesic, Antitumor and Antioxidant potential of total saponins from *Nigella glandulifera* seeds. *J Evid Based Complementary Altern Med.* 2013;2013:827230. doi: 10.1155/2013/827230
 28. Shahzad M, Yang XD, Aseim MBR, et al. Black seed oil ameliorates allergic airway inflammation by inhibiting T- cell proliferation in rats. *Pulm Pharmacol Ther.* 2009;22(1):37-43. doi: 10.1016/j.pupt.2008.11.006
 29. Andlousi AB, Martinear LC, Vallerand P, et al. Multiple molecular targets underlie the antidiabetic effect of *Nigella sativa* seed extract in skeletal muscle adipocyte and liver cells. *Diabetes Obes Metab.* 2010;12(2):148-157. doi: 10.1111/j.1463-1326.2009.01131.x
 30. Kaseb AO, Chinnakannu K, Chen D, et al. Androgen receptor and E2F-1-targeted thymoquinone therapy for hormone-refractory prostate cancer. *Cancer Res.* 2007;67(16):7782-7788. doi: 10.1158/0008-5472.CAN-07-1483
 31. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics resistant bacteria. *Acta Microbiol Pol.* 2000;49(1):63-74.
 32. Swamy SMK, Tan BKH. Cytotoxic and immunopotentiating effects of ethanolic extract of *Nigella sativa* L. seeds. *J Ethnopharmacol.* 2000;70(1):1-7. doi: 10.1016/S0378-8741(98)00241-4
 33. Kanter M, Meral I, Dede S, et al. Effects of *Nigella*

- sativa* L. and *Urtica dioica* L. on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in ccl4- treated rats. *J Vet Med.* 2003;50(5):264-268. doi: 10.1046/j.1439-0442.2003.00537.x
34. Kooti W, Noohi ZH, Ahvazi NS, Samabi MA, Larky DA. Phytochemistry Pharmacology and therapeutic uses of black seed (*Nigella sativa*). *Chi J Nat Med.* 2016;14(10):0732-0745. doi: 10.1016/S1875-5364(16)30088-7
 35. Ameedy TH, Omaran R. Antimicrobial activity of *Nigella sativa* extract against some bacteria and fungal species. *Journal of University of Babylon of Pure and Applied Sciences.* 2019;27(1):277-286
 36. CLSI performance standard for antimicrobial disk susceptibility test, approved standard. 7th ed. CLSI document M02-A 11. Clinical and laboratory standard institute. 950, west valley road, suite 2500, Wayne Pennsylvania 19087, USA. 2012.
 37. CLSI methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, approved standard. 2012. 9th ed. CLSI document M07- A9. Clinical and laboratory standard institute. 950, west valley road, suite 2500, wayne Pennsylvania 19087, USA .
 38. Harbone JB. Phytochemical methods: a guide to modern techniques of plant analysis. Chapman and hall publications, London. 1998.
 39. Evans WC, Trease GE. 11th ed. Bailliere, Tindall, London. 1989.
 40. Mahlo SM, Chauke HR, Mc Graw LJ, Eloff JN. Antioxidant and antifungal activity of selected plant spp used in traditional medicine. *J Med Plant Res.* 2013;7(33):2444-2450.
 41. Ahmad I, Aquil F. *In vitro* efficacy of bioactive extracts of 15 medicinal plants against ESbetaL-producing multidrug resistant enteric bacteria. *Microbiol Res.* 2007;162(3):264-275. doi: 10.1016/j.micres.2006.06.010
 42. Joray MB, Rollan MDR Ruiz GM, Palacios SM, Carpinella MC. Antibacterial activity of extracts form plants of central Argentina- isolation of active principle from *Achyrocline satureioides*. *Planta Med.* 2011;77(1):95-100. doi: 10.1055/s-0030-1250133
 43. Mambe FT, Igor K, Beng VP, Kuete V. Antibacterial acidity of methanol extracts form *Alchornea cordifolia* and four other cameroonian plants against MDR phenotypes. *Journal of Taibah University Medical Sciences.* 2016;11(2):121-127. doi: 10.1016/j.jtumed.2015.12.001
 44. Jagessar RC, Alleyne R. Atimicrobial potency of aqueous extracts of leaves of *Terminalia catappa*. *ARInt.* 2011;1(3):362-371.
 45. Sahina N, Ahmad S, Rasool SA. *In vitro* antibacrtial activity of the extracts derived from *Terminalia catappa*. *Res J Micribiol.* 2007;2(2):180-184. doi: 10.3923/jm.2007.180.184
 46. Babayi H, Kolo I, Okogun JI, Ijah UJJ. The antimicrobial activities of methanol extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokimistri.* 2004;16(2):106-111. doi: 10.4314/biokem.v16i2.32578
 47. Chibane LB, Forquet V, Lanteri D, et al. Antibacterial properties of polyphenols: characterization and QSAR (Quantitative structure activity relationship models). *Front Microbiol.* 2019;10:829. doi: 10.3389/fmicb.2019.00829
 48. Zhang XR, Kaunda JS, Zhu HT, Wang D, Yang CR, Zhang YJ. The Genus *Terminalia* (Combretaceae): An Ethnopharmacological Phytochemical and Pharamacological review. *Nat Prod Bioprospect.* 2019;9:357-392. doi: 10.1007/s13659-019-00222-3
 49. Shinde SL, Junne SB Wadje SS, Baig MMV. Diversity of antibacterial compounds of *Terminalia* spp. (Combretaceae). *Pak J Biol Sci.* 2009;12(22):1483-1486. doi: 10.3923/pjbs.2009.1483.1486
 50. Sanghvi R, Venkatalakshmi P, Brinda P. Antibacterial activity of *Terminalia catappa* bark against some bacterial pathogens. *World J Pharm Sci.* 2015;4(9):987-992.
 51. Neelavathi P, Venkatalakshmi P, Brindha P. Antibacterial activity against aqueous and ethanolic extracs of *Terminalia catappa* leaves and bark against some pathogenic bacteria. *Int J Pharm Pharm Sci.* 2013;5(1):114-120
 52. Khan AR, Kour K. Wide spectrum antibacterial activity of *nigella sativa* seeds L. *IOSR Journal of Pharmacy.* 2016;6(7):12-16. doi: 10.9790/3013-06721216
 53. Benlafya K , Karrouchi K, Charkaoui Y, Karbane ME, Ramli Y. antimicrobial activity of aqueous, ethanolic, methanolic cyclohexanic extracts and essential oil of *Nigella sativa* seeds. *J Chem Pharm Res.* 2014;6(8):9-11.
 54. Amina B, Rachida A. Moleuclar Composition and antibacterial effect of essential oil of *Nigella sativa*. *Afr J Biotechnol.* 2013;12(20):3006-3012.
 55. Singh C, Das SS , Singh G, Schuffc De lampasona MP, Catalan CAN. compositon, *in vitro* antioxidant and antimicrobial activities of essential oil and oleoresins obtained from black cumin seeds (*Nigella sativa* L.). *Biomed Res Int.* 2014;9:18209. doi: 10.1155/2014/918209
 56. Sudhir SP, Rohitkumar, S, Verma HN. Study of antimicrobial potential, chemical composition and free radical scavenging property of *Nigella sativa* seed cold pressed oil and N-hexane extract from different geographies. *Int J Pharm Biol Sci.* 2017;7(2):131-144.
 57. Zuridah H, Fairuz ARM, Zakri AZH, Rahim MNA. *In vitro* antibacterial acitivity of *Nigella sativa* against *Staphylococcus aureus*, *Pseudomonas aeroginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Bacillus cereus*. *Asian J Plant Sci.* 2008;7(3):331-333. doi: 10.3923/ajps.2008.331.333
 58. Tpcagic A, Zeljkovi, SC Karalija E, Galijasevic, SC, Sofic E. Evaluation of phenolic profile, enzyme inhibition and antimicrobial activity of *Nigella sativa* loaded seed extracts. *Bosn J Basic Med Sci.* 2017;4:286-294. doi: 10.17305/bjbms.2017.2049
 59. Sahak MKZ, Kabir N, Abbas G, Daraman S, Hashim NH, Adli DSH. Role of *Nigella sativa* and its active constituents in learning and memory. *Evid Based Complementary Altern Med.* 2016. doi: 10.1155/2016/6075679
 60. Mogana R, Adhikari A, Tzas MN, Ramliza R, Wiart C. Antibacterial activity of the extracts fractions and

- isolated compounds from *Canarium patentinerium* Miq. Against bacterial clinical isolates. *BMC Complement Altern Med.* 2020, 20:55. doi: 10.1186/s12906-020-2837-5
61. Kloucek P, Polesny Z, Svobodova B, Vlokova E, Kokosla L. Antibacterial screening of some Peruvian medicinal plants used in calleria district. *J Ethnopharmacol.* 2005;99:309-312. doi: 10.1016/j.jep.2005.01.062
62. Akharaiyi FC, Ilori RM, Adesida JA. Antibacterial effect of *Terminalia catappa* on some selecte pathogenic bacteria. *Int J Pharm Biomed Res.* 2011;2(2):64-67.
63. Cock IE, Vuuren SFV. A composition of the antimicrobial activity and toxicity of six Combretum and two *Terminalia* spp from southern Africa. *Phcog Mag.* 2015;11(41):208-218. doi: 10.4103/0973-1296.149740
64. Toghueo R, Boyom FF. Enophytic fungi from *Terminalia* species. A comprehensive review. *J Fungi.* 2019;5(2):43. doi: 10.3390/jof5020043
65. Opara FN, Angufoho HU, Okechukwu RI, Mgbemena IC, Akuyobic O, Adjew A. A preliminary phytochemical screening and antibacterial activity of leaf extracts of *Terminalia catappa*. *JETEAS.* 2012;3(3):424-428.
66. Mbengui RD, Guessennd NK, M'Boh GM, et al. Phytochemical screening and study of comparative antibacterial activity of aqueous and alcoholic extracts of the leaves and barks of *Terminalia catappa* on multi resistant strains. *J Applied Biosci.* 2013;66:5040-5048. doi: 10.4314/jab.v6i0.95000
67. Muhammad A, Mudi SY. phytochemical screening and antimicrobial activity of *Terminalia catappa* leaf extracts. *Biokemistri.* 2011;23(1):35-39.
68. Ahmed MS, VeerabhadraSwamy BM, Dhanpal PGR, Chandrashekara VM. Antidiabetic activity of *Terminalia catappa* L. leaf extracts in alloxan-induced diabetic rats. *IJPT.* 2005;4(1):36-39.
69. Dukes A. Phytochemical and ethnobotanical database. 2008:11.
70. Mndloi S, Mishra R, Varma R, Varugheese B, Tripathi. A study on phytochemical and antifungal activity of leaf extracts of *Terminalia catappa*. *J Int J Pharm Biosci.* 2013;4(4):1385-1393.
71. Reddy HS, Al-kalbani AS, Al-Rawahi AS. Studies on phytochemical screening and GC-MS characterization, antimicrobial, antioxidant assay of black cumin seeds and Senna alexandria solvent extracts. *Int J Pharm Sci.* 2018;9(2):490-497.
72. Desai SD, Saheb SH, Das KK, Haseena S. Phytochemical analysis of *Nigella sativa* and its antidiabetic effect. *J Pharm Sci Res.* 2015;7(8):527-532.
73. Yadav A, Yadav M, Kumar S, Yadav JP. Bactericidal effect of *Acacia nilotica*: *In vitro* antibacterial and time kill kinetic studies. *International Journal of Current Research.* 2015;7(11):22289-22294.
74. Ngouana TK, Mbouna CDJ, Kuipou RMT, et al. Potent and synergistic extract combination from *Terminalia catappa*, *Terminalia mentaly* and *Monodora tenuifolia* against pathogenic yeasts. *Medicines.* 2015;2(3):220-235. doi: 10.3390/medicines2030220