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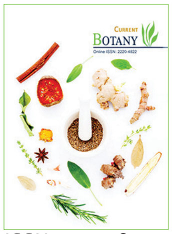

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Antibacterial evaluation of *Dalbergia latifolia* (Roxb.) liquid smoke used for the treatment of bovine mastitis

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ABSTRACT

The indigenous people of the area have long utilized medicinal plants to treat bovine mastitis. Traditional ethnoveterinary healers used plant-based techniques to treat diseased cows. *Escherichia coli* and Coagulase-negative *Staphylococci* (CoNS) bacteria infect the udder and cause mastitis, which lowers milk production. In this work condensed smoke of heartwood of *Dalbergia latifolia* was produced by pyrolysis at 500 °C for 8 hr and filtered with Whatman No.1. The liquid smoke was analysed with GC-MS and found 23 bioactive phytocompounds that have antibacterial activity of mastitis causing bacteria *E. coli* and CoNS. The antibacterial activity was carried out using disc diffusion methods with three different concentrations of liquid smoke which were 150 µL/mL, 300 µL/mL and 600 µL/mL of distilled water. The maximum inhibition average of 21.00 mm at 600 µL/mL treatment *E. coli* (S1) and 28.33 mm at 600 µL/mL on *E. coli* (S2), the positive control average value of 15.88 mm and 0.00 mm in *E. coli* (S1) and *E. coli* (S2) respectively. The maximum inhibition of 20 mm of CoNS average value 2 of 4.66 mm was estimated at 600 µL/mL treatments. The Ampicillin antibiotics disc was used to have a positive control for all the treatment. The liquid smoke of *D. latifolia* has higher antibacterial properties to cure mastitis disease in dairy cows.

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INTRODUCTION

The application of traditional knowledge, beliefs, abilities, practices, and methods to treat animal illnesses using locally accessible flora is known as ethnoveterinary medicine. Traditional animal medicine, or EVM, offers affordable substitutes for allopathic medications. Traditional EVM offers cheaper, simpler, and more environmentally friendly substitutes for pricey antibiotics (Xiong & Long, 2020). According to World Health Organization (WHO) reports, approximately 80% of the world's population is dependent on plants for their health, people that live in rural regions (Prakash *et al.,* 2021). Animal husbandry is well ingrained in the cultural traditions of many ethnic communities that justify the popularity of indigenous knowledge, belief and practices in maintaining the health and well-being of cattle (Selogatwe *et al.*, 2021). Dairy is India's leading agricultural commodity, also according to the Food and Agriculture Organization (FAO) India has transformed from a country with milk deficient to a leading milk producer. The Dairy animals are a regular source of income and women play a crucial role in milk production in rural area (Pandey *et al.*, 2021). Bovine Mastitis to be significant obstacle to achieve production goals, as the affected quarters may have 30 % productivity, leading to overall production loss of about 15 % in dairy animals (Solanki *et al.*, 2022). Mastitis is the most common disease affecting dairy cattle, caused by bacterial microorganisms a that increased somatic cell count (SCC), physical changes in milk, which decline milk production (Badawy *et al.*, 2022). Around 150 pathogenic bacterial species responsible for udder inflammation were identified (Grudlewska-Buda *et al.*, 2021). Mastitis causing bacterium divided into two classes contagious pathogens and environmental pathogens. Cogulase-negative *staphylococci* (CoNS), *Streptococcus agalactiae*, and *S. dygalactiae* are contagious pathogens that were spread among milch cows through milking instruments or other means. *Escherichia coli*, *S.uberis*, *Klebsiella pneumoniae* and Coagulase-negative *Staphyalococcus* are known as environmental pathogens they are mainly found in soil, water and beds, and are spread through contact with udder (Xu *et al.*, 2022). The environmental pathogenic microorganisms including coliforms are the most contribution to the clinical mastitis causing severe inflammation with clinical signs in dairy cows, which however sometimes immune systems heal itself (Sharifi *et al.*, 2018). The subclinical mastitis is coined as when the symptoms are not easy to observe but there is a decrease in milk production. Subclinical mastitis changes the colour of the milk, it's also may have pathogenic microbes that leads to disease. Subclinical mastitis made more losses than clinical mastitis and its contiguous risk of spreading in a herd (Kaczorowski *et al.*, 2022).

Holstein cows' detection with clinical mastitis caused by *E. coli* had a decline in milk yield of about 15%, which is considered an average milk loss of about 200 litters per cow during the 305 days of lactation. Coliform bacteria are the most common pathogenic microbe responsible for mastitis (Ahmed *et al.*, 2021). *E. coli* infection affects an inflammatory immune response in the mammary gland, regulates the recruitment of neutrophils and macrophages to the infectious site. Neutrophils, actively predominant immune cells, and attempt to phagocytose bacteria also cause damage to tissues and decrease the milk production rate (Goulart & Mellata, 2022). The *E. coli* is a Gram-negative, anaerobic, rod-shaped bacterium. *E. coli* has natural resistance to soil and cow's intestines. The bacterium is commonly found in water, manure and bedding (Grudlewska-Buda *et al.*, 2021). The Bovine mastitis caused by *E. coli* cases has risen in recent years. The antibiotics are commonly used to treat *E. coli* infected mastitis, achieving improved effectiveness in animal husbandry and veterinary clinics: they were research shown that *E. coli* can quickly develop resistance to frequently used antibiotics and with rapid genetic variation (Xu *et al.*, 2022). *Staphylococci* species are classified into two groups based on coagulase production capability – coagulase-positive *staphylococci* (CoPS) and coagulase-negative *Staphylococci* (CoNS). The CoNS were thought to be less pathogenic and noted only in the sub-clinical sample of mastitis. The mastitis is also nowadays developed with multi-drug resistance in dairy animals, primarily due to excessive use of antibiotics. *Staphylococci* species are also most common bacteria associated with intra-mammary infections (Solanki *et al.*, 2022). Clinical and Subclinical mastitis occurs with *staphylococcus* species being the causative in both and other pathogens may also play a role. Specifically, CoNS – such as *S. caprae*, *S. epidermidis*, *S. xylosus*, *S. chromogens*, *S. simulans* are most commonly associated with subclinical mastitis disease, whereas *Staphylococcus aureus* tend to cause clinical mastitis more frequently (Nelli *et al.*, 2022). Antimicrobial therapy using natural products is growing and some recent studies investigating the antimicrobial effects of plant-based medicine on pathogens from bovine mastitis. Mostly these studies focused on plants native to specific geographic regions (Arbab *et al.*, 2022). The increasing potential of antibiotic resistance of pathogens with high cost and more side effects of drugs have lead the interest of researchers for the potential discovery of useful compounds (ul Hassan *et al.*, 2014). *Dalbergia latifolia* (Roxb.) tree is an important timber tree species belonging to the Fabaceae family internationally known as Indian Rosewood. It is used to manufacture furniture and other decorative products. In India, it's mostly located in the Western Ghats, commonly called in different regions Shisham, Kali shisham or Bombay Blackwood. Traditional medicinal use of *D. latifolia* tree parts for treating respiratory disease, skin disease, and digestive problems (Dhandapani *et al.*, 2020) and Latifolin compound found in the heartwood of *D. Latifolia* (Deshmukh *et al.*, 2021). The main compound constituents of *D. latifolia* bark are Phenolic, Flavonoids, carbohydrates, glycosides, tannins, and amino acids (Khalid *et al.*, 2011). The heart wood contained neoflavanoid, dalcriodon and latinone and Isoliquiritigenin (Dhandapani *et al.*, 2020). The oil extraction from the firing heart wood of *D. latifolia* is used to cure skin diseases (Deshmukh *et al.*, 2021). The bioactivities exhibited by extracts from plants are antioxidant, cryoprotective, anticancer and antimicrobial properties, it also mentions particularly benzofurans that are noted for potential health benefits (Liu *et al.*, 2018).

The present study screens biocompounds of *D. latifolia* liquid smoke to evaluate their antibacterial activity and increase focus on tackling antimicrobial resistance by finding alternative regimens.

MATERIALS AND METHODS

Field Survey

The collection of the data for the curing of mastitis diseases in the cow with the help of traditional plant-based medicine, the interaction of the healer who has traditional knowledge of the EVM. We are visited in the Dharampur Taluka of Valsad district, Gujarat, India. The total number of 26 healers suggested that plants *Dalbergia latifolia* (Roxb.) used in direct heart wood smoke treatment applied to the udders of cows to cure mastitis.

Biomass Pyrolysis

D. latifolia heartwood has been collected from the Dharampur forest, fully matured (life cycle completed plant), dried trunk wood and cut into small pieces and ground into powder form for pyrolysis. The powdered sample was oven dried at 50 °C for the 24 hr, the low moisture content in the sample accelerates the rate of the pyrolysis during the smoke production, the liquid smoke production by the pyrolysis 30 L capacity reactor at 500 °C for 8 h, the LPG burner used for the heating and temperature controlled by the gas flow controller. The 5 kg of dried sample material was loaded into the reactor and smoke generated from the reactor passed through the tube cyclone to separate the heavy tar content and ash of the wood. The smoke was condensed with the help of the water inlet and outlet condenser using water as coolant and finally obtained condensed smoke in the form of liquid. Some tar like residue left in the reactor was charcoal and the obtained liquid smoke was stored at 4 °C in the refrigerator for further use (Mansur *et al.*, 2023).

GC-MS Analysis of the Liquid Smoke

GC-MS analysis of liquid smoke extracts was carried out using the Perkin-Elmer Clarus 680 system (Perkin-Elmer, Inc. U.S.A) equipped with a fused silica column, packed with Elite-5MS capillary column (30 m in length x 250 μm in diameter x 0.25 μm in thickness). Pure helium gas (99.99%) was used as the carrier gas at a constant flow rate of 1 mL/min. For GC-MS spectral detection, an electron ionization energy method was adopted with ionization energy of 70 eV (electron Volts) with 0.2 s of scan time and fragments ranging from 40 to 600 m/z.

the injection quantity of 1 μL was used (spilt ratio 10:1), and the injector temperature was maintained at 250 °C (constant). The column oven temperature was set to 50 °C for 3 min, raised to 10 °C, and the final temperature was increased to 300 °C for 10 min. The contents of phytochemicals present in the last samples were identified based on a comparison of their retention time (min), peak area, peak height and mass spectral patterns with that spectral database of authentic compounds stored in the National Institute of Standards and Technology (NIST) library.

Collection of the Milk from Infected Cow and Isolation of Bacteria

The two cow's species (Holstein) were identified with infected from mastitis and from both cow's infected milk (Sample 1 - white coagulated milk and Sample 2 - has coagulated blood presence in the milk) samples were collected from the Dulsad, Bodlai, village of the Valsad district according to the guidelines of the National Mastitis Council (Dohoo *et al.*, 2010). Sample 1 (S1) and sample 2 (S2) milk were cultured on MacConkey agar and nutrient agar, the inoculated plates were incubated at 37 °C for 18 to 24 hours. Pathogens were identified by their characteristic appearances on the media, gram stain, motility and biochemical reaction as per standard laboratory protocol. The pathogens were isolated from S1 were *Escherichia coli* and S2 were *Escherichia coli* and CoNS.

Preparation of the Concentrations of the Liquid Smoke

The liquid smoke of plant *D. latifolia* was centrifuged at 10000 rpm for 15 min, and the supernatant was collected and the pellets were discarded. The collected supernatant was filtered through the syringe filter, and pure filtered liquid smoke was used for the preparation of the different concentrations for the antibacterial susceptibility testing. The three different concentrations 150, 300, and 600 μL were mixed in 1 mL distilled water of each concentration in the separate Eppendorf tube. Antimicrobial susceptibility testing was finished by Kirby-Bauer plate dispersion strategy on Muller Hinton agar. The different doses were added to the blank disc, Whatman filter paper, for antibacterial testing of the condensed liquid smoke.

Analysis

All experiments were performed in the laboratory and the presented data are the average mean and standard error with the three replicates. All data subjected to the one-way ANOVA differences in the given sample with DMRT and P- value of 0.05 considered. The SPSS ver. 27.0 IBM USA was used.

RESULTS

GC-MS Analysis of the Liquid Smoke

The smoke extract of both plants *Dalbergia latifolia* (Roxb.) subjected to GC-MS analysis to investigate the volatile bioactive compounds that may also be responsible for the medicinal and pharmaceutical properties of this plant. The GC-MS chromatograms represent the constituents profiling of the extracted condensed smoke, the sharp peaks indicated the higher bioactive compounds and the lower broad peaks indicate the low biochemical compounds. The 23 bioactive compounds were identified respectively and reported to have medicinal properties such as anti-inflammatory, antioxidant, antiseptic and antimicrobial effects. The detected volatile compounds may have synergistic effects on antibacterial properties. The identified bio compounds are shown in Table 1 chromatogram graph (Figure 1).

Table 1: The GC-MS based identified biocompounds from liquid smoke of the *Dalbergia latifolia*

Phytochemicals identified	Retention Time	Peak height	Area %	Molecular weight g/mol	Molecular formula
Fomic acid	2.398	33961	2.84	46.025	CH_2O_2
Acetic acid	2.625	181935	13.78	60.05	$C_2H_4O_2$
2- Propanone, 1-hydroxy	3.042	500216	20.91	74.0785	$C_3H_6O_2$
Acetic Acid, (acetyloxy)-	4.309	39209	4.48	118.0880	$C_4H_6O_4$
Succindialdehyde	4.495	43340	4.32	86.09	$C_4H_6O_2$
Di (3-Methylbutyl) amin	7.274	8541	0.53	157.2963	$C_10H_{23}N$
1H-Imidazole, 4, 5-dihydro-2-methyl-	7.437	19513	2.13	84.1197	$C_4H_8N_2$
1,2-Cyclopentanedione	7.825	21105	1.76	98.10	$C_5H_6O_2$
2-Cyclopenten-1-one, 2-hydroxy-3-methyl-	10.787	41509	2.88	112.1265	$C_6H_8O_2$
1,2-Ethanediol, 1-(2-furyl)-2-phenyl-	11.679	13653	1.41	128.13	$C_6H_8O_3$
Phenol, 2-methoxy-	12.618	40785	5.82	124.1372	$C_7H_8O_2$
Cyclopropyl carbinol	12.869	56558	4.52	72.1057	C_4H_8O
Creosol	15.769	36838	3.99	138.1638	$C_8H_10O_2$
1,4:3,6-Diahydro-.alpha.-d-glucopyranose	16.459	34524	2.83	144.12	$C_6H_8O_4$
3,4-Anhydro-d-galactosan	16.743	11645	0.87	144.12	$C_6H_8O_4$
2,3-Anhydro-d-mannosan	16.857	20229	1.62	144.12	$C_6H_8O_4$
Phenol, 4-ethyl-2-methoxy-	18.226	19461	1.80	152.1904	$C_9H_{12}O_2$
Phenol, 2,6-hydroxytoluene	20.225	44436	3.37	220.35	$C_{15}H_{24}O$
3,5-Dimethoxy-4-hydroxytoluene	22.721	25697	2.27	168.1898	$C_9H_{12}O_3$
.beta.-D-Glucopyranose, 1,6-anhydro-	23.926	57935	11.20	162.1406	$C_6H_10O_5$
2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)	24.781	17730	2.72	180.2005	$C_10H_{12}O_3$
Idosan triacetate	25.114	20446	2.08	288.25	$C_{12}H_{16}O_8$

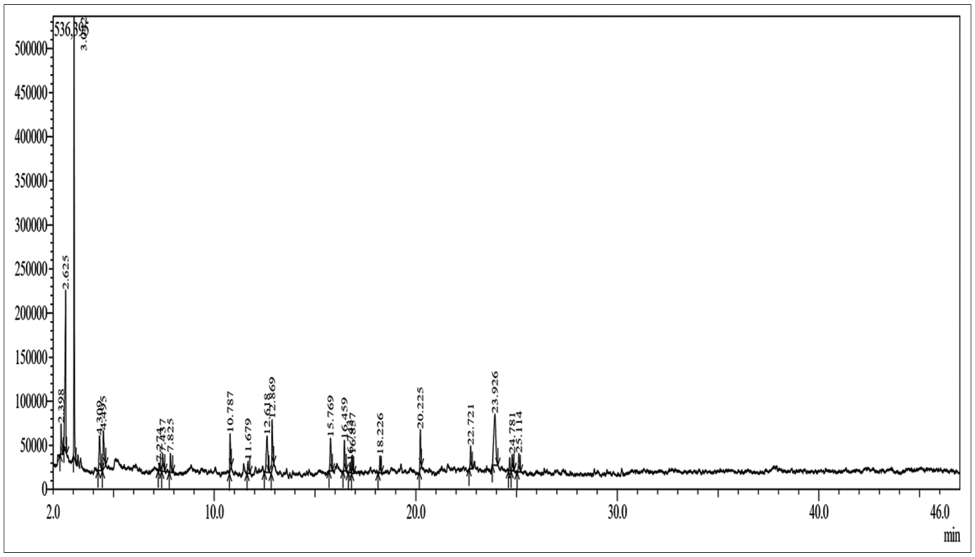

Figure 1: The GC-MS analysed chromatogram of the liquid smoke of *Dalbergia latifolia*

Antibacterial Activity of Liquid Smoke

The study investigates the *in vitro* antibacterial activity of minimum inhibitory concentration (MIC) and Maximum Bacterial concentration of has been tested with *D. latifolia* condensed liquid smoke, the three concentrations 150, 300 and 600 µL/mL were used against mastitis diseased causing bacteria *E. coli* (S1), *E. coli* (S2) and CoNS. The liquid smoke has antibacterial properties to inhibit the bacterial culture growth. The maximum inhibition zone was estimated an average value of 21.00 mm in the higher dose 600 µL/mL treatment in the *E. coli* (S1), and the lowest inhibition zone was estimated at an average value of 14.33 mm in the low dose 150 µL/mL treatment. The Ampicillin antibiotic drug was used as positive control and the inhibition zone was estimated at an average value of 15.83 mm compared with all treatments. The mastitis disease causes other bacteria CoNS in cows, the liquid smoke is applied to for testing of antibacterial test. The cultured bacterial inhibition zone was estimated and finds that maximum zone of the inhibition average value was 24.66 mm in the 600 µL/mL treatment, and the average value of inhibition of the lowest dose was estimated the 19.33 mm in the 150 µL/mL treatment, the smoke extract produced anti inhibition zone against CoNS bacteria. The ampicillin antibiotic disc was used as a positive control, and an average value was estimated at 30.66 mm which was higher than each treatment of the liquid smoke (Figure 2 & Table 2).

The liquid smoke biochemical properties have significant potential for the development of antibiotic drugs for the curing of mastitis disease in cows and other veterinary antibiotic applications.

DISCUSSION

The ethno-veterinary traditional medicine was used to treat mastitis diseases in the cows, the local healers used the heartwood smoke of the *D. latifolia* for direct treatment on the udder. The present research work demonstrated that liquid smoke biocompounds properties with the help of GC-MS, the 23 bioactive compounds were detected in the analysis and the liquid smoke was used for the antibacterial test on the two bacterial strains *E. coli* and CoNS. The similar biocompounds were reported Wang *et al.* (2018) volatile biocompounds in 14 plants sample and total 213 phytochemical constituents were identified by the analysis of the GC-MS, the identified compounds have Unique's properties such as terpenoids (monoterpenes and sesquiterpenes derivatives) and phenols. Teoh *et al.* (2023) studied the phytochemical compounds in the *Lygodium microphyllum* as many classes include the phenolics, terpenoids, flavonoids, steroids, microlides, lipids and vitamins, and other some hydrocarbons also detected in the plants crude sample that had medicinal properties for the drug development. Mathe *et al.* (2024) reported the two medicinally important plants *Sutherlandia fruitesens* and *Withamia somnifera* for their phytochemical constituents in the leaves sample, the chromatogram identified many secondary metabolites such as steroids, alkaloids, quinones, cardiac glycosides, terpenoids and coumarins. The identified compounds are used as traditional medicinal drugs. Raza *et al.* (2024) reported the essential oil constituents in three different plants *Salvia officinallis*, *Canabis sativa* and *Laurus nobilis*, the identified composition was transcaryophylline, alpha pinene and viridifloral in hemp species, myrcene and limonene and other beta caryophyllene in the sage species, the phytol and beta myrecene identified in the laurel oil. Sehim *et al.* (2023) analysed bioactive compounds in the *Hibiscus sabdariffa* for antibacterial activity, the various compounds were identified by the GC-MS analysis of the hexadecanoic acid, hexadecenoic methyl ester, oleic acid, and 3 hydroxypropropyl ester etc. the identified compounds showed antibacterial activity in *E. coli* and *Pseodomonas* strains. Gad *et al.* (2020) studied the Astragalus species which have many antioxidant phytoconstituents such as octanal, hexanal, 2 ethyl hexanal, terpineol, butyl decanoate, trico sane and vanillin, etc.

The present work indicated the antibacterial activity of the liquid smoke on the *E. coli* (S1), *E. coli* (S2) and CoNS, the

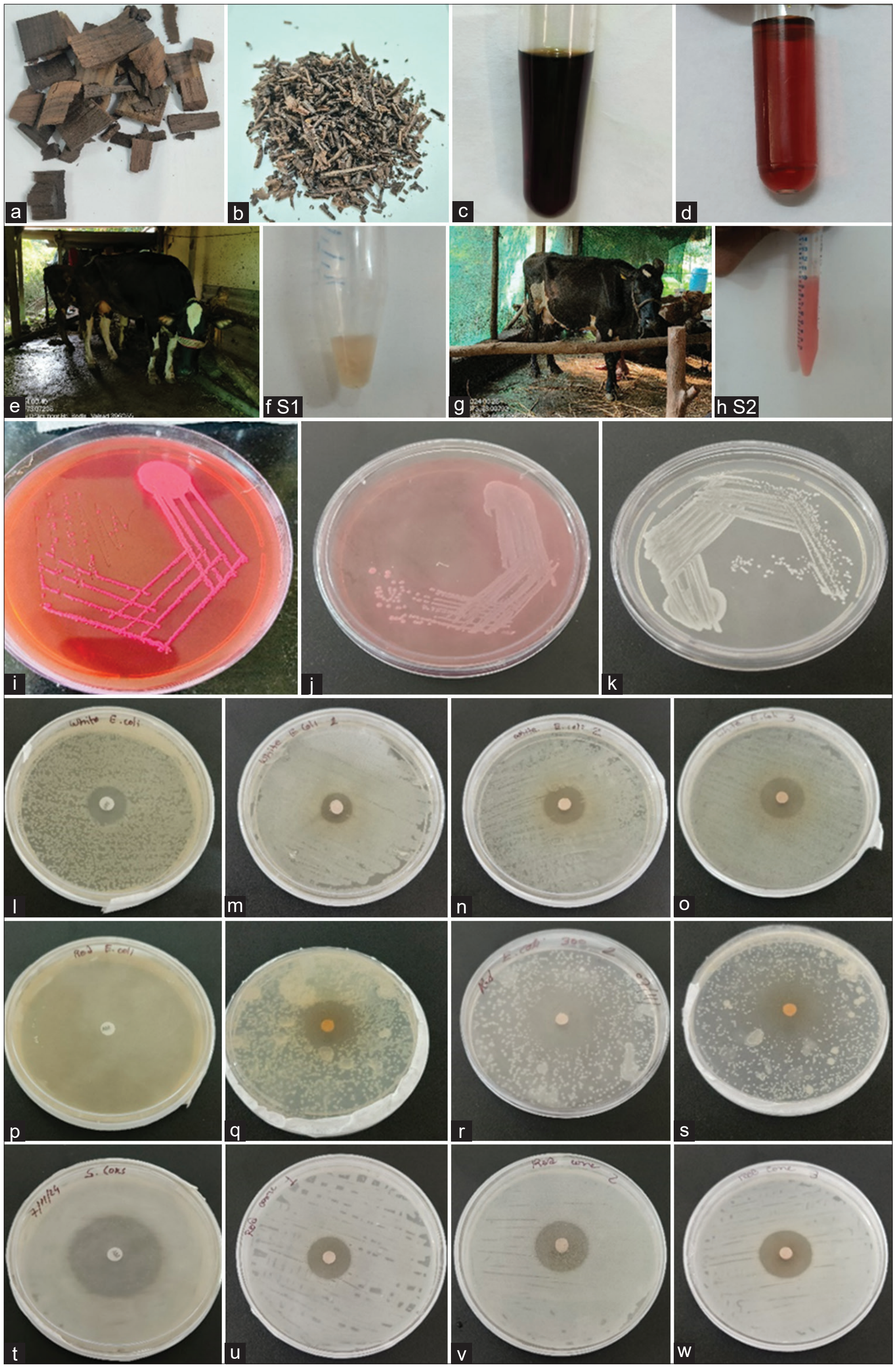

Figure 2: a) *D. latifolia* heartwood, b) Wood husk, c) Pure condense liquid smoke, d) Filtered liquid smoke, e) Mastitis infected cow (Holstein), f) Milk sample - 1 (S1), g) Mastitis infected cow (Holstein), h) Milk sample - 2 (S2), i) Pure bacterium isolation *E. coli* (S1), j) Pure bacterium isolation *E. coli* (S2), k) Pure bacterium isolation CoNS, l) Ampicillin antibiotic disc *E. coli* (S1), m) 150 μL/mL, n) 300 μL/mL, o) 600 μL/mL, p) Ampicillin antibiotic disc *E. coli* (S2), q) 150 μL/mL, r) 300 μL/mL, s) 600 μL/mL, t) Ampicillin antibiotic disc CoNS, u) 150 μL/mL, v) 300 μL/mL and w) 600 μL/mL dose of liquid smoke of *D. latifolia*

maximum inhibition zone of 21.00 mm of the *E. coli* (S1) and lower mean value was 15.83 mm, the maximum inhibition zone 28.33 mm of the *E. coli* (S2) and the minimum zone was 24.00 mm at lower treatment 150 μL/mL. Wang *et al.* (2021) tested antibacterial activity of ginger essential oil against *E. coli* and *Staphylococcus aureus* and measured the diameter of the inhibition zone. The *S. aureus* measured inhibition zone was 17.1 mm and *E. coli* measured inhibition zone 12.3 mm. The

Table 2: Data represented in table are antibacterial test in different concentrations of liquid smoke D. latifolia on the *E. coli* (S1), *E. coli* (S2) and CoNS

Treatment in (μL/mL) DW	*E. coli* (S1)	*E. coli* (S2)	CoNS (S2)
Control (AMP)	15.83±0.166[ab]	0.00±0.00	30.66±0.33[c]
150	14.33±0.33[a]	24.00±1.00[b]	19.33±0.33[a]
300	16.66±0.88[b]	25.66±1.20[b]	23.66±1.20[b]
600	21.00±0.57[c]	28.33±2.40[b]	24.66±0.66[b]
Sum of Sq.	73.79	275.78	196.25
Mean Sq.	24.57	87.07	65.41
Frequency	26.215	14.073	41.316
Significance	0.192	0.204	0.066

ginger essential oil minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were MIC for *S. aureus* 1.0 μL/mL and MBC 2.0 μL/mL and MIC dose E. coli 2.0 μL/mL and MBC 4.0 μL/mL. The present research work antibacterial test of *E. coli* (S2) was tested with liquid smoke and produced a higher antibacterial inhibitory zone in mastitis causing bacteria, the maximum inhibition zone was estimated the average value of 28.33 mm in the higher dose of 600 μL/mL of the liquid smoke, and the 24.00 mm zone was estimated in the low dose 150 μL/mL treatment compared to the control. The positive antibiotic control inhibition zone 0.00 mm didn't show any antibacterial activity of the mastitis causing bacteria. The *E. coli* strains show susceptibility and sensitivity against different antibiotic drugs and plant wood extracts.

This research work indicated that the antibacterial activity of the liquid smoke on CoNS was a maximum inhibition zone of 24.66 mm recorded, and a minimum inhibition zone of 19.33 mm compared to the other treatments. Srichok *et al.* (2022) *Ocimum tenuiflorum* showed significant antibacterial effects against Coagulase-negative *Staphylococci* (CoNS) and however it did not exhibit antibacterial activity against gram-negative bacteria *E. coli*. The MIC values for *Ocimum tenuiflorum* extract ranged from 3.9 to 31.2 μL/mL against gram-positive bacteria. The MBC values show against Coagulase-negative *Staphylococci* (CoNS) is 15.6 μL/mL. Šukele *et al.* (2023) used *Tenacentum vulgare* flower and leaves extract against *S. aureus* and *E. coli* pathogenic bacteria MIC 14.8 mm and 8.8 mm with 70% Ethanolic extract. Elisha *et al*. (2017) studied some medicinal plant extracts against *E. coli* with *Cremaspora trifloral* MIC of 0.05 mg/mL, Maesa lanceolata MIC of 0.04 mg/mL and *Hypericum roperianum* 0.13 mg/mL. Kebede *et al.* (2021) studied the methanolic extracts from *Euphorbia depauperata* 15 mm, *Cirsium englerianum* 22 mm and *Discopodium penninervium* 27 mm shows inhibition zone respectively. Boonkusol *et al.* (2022) studied *Caesalpinia sappan* L. aqueous and ethanol extract antibacterial activity against Coagulase-negative *Staphylococci* (CoNS) isolate from subclinical mastitis were ethanol extract exhibited better antibacterial activity to the aqueous extract. The *C. sappan* ethanol extracted showed 21.0 mm maximum inhibitory zone whereas the aqueous extract had 14.7 mm against bovine mastitis CoNS.

The present research work identified the 23 bioactive compounds in the *D. latifolia* that have medicinal properties for the treatment of the mastitis disease in the cow, and liquid smoke showed the antibacterial activity in the mastitis causing bacteria.

CONCLUSION

The plant based traditional medicine was used by the local healers and traditional based information was tested in the laboratory for the curing of mastitis disease. The pyrolysis of *D. latifolia* wood husk created liquid smoke that contained biocompounds such as carboxylic acids, phenolic compounds, alkaloids, and flavonoids. These biocompounds have antibacterial properties to inhibit the two bacterial strains *E. coli* (S1) and *E. coli* (S2) and CoNS. The result indicated that *D. latifolia* liquid smoke used to cure mastitis disease in the infected cows, and the bioactive compounds have properties to prepare pharmaceutical medicines for the curing of the disease in cows.

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AUTHORS' CONTRIBUTION

TP performed research work, collected fieldwork data and laboratory analysis. KP provides guidance for field work surveys. RP performs antibacterial activity. All authors agree to publish this work.

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