

Anti-microbial Activity and GC-MS Analysis of Leaves Extracts of *Butea Monosperma* (Lam.) Taub

Surabhi Ambastha*, Amit Patnaik, Vinay Oraon, and Latika Sharan

Ranchi University, Ranchi-834 008, India

*E-mail: surbhiambastha125@gmail.com

ABSTRACT

Butea monosperma (Lam.) Taub is a well-known medicinal plant belonging to the family Fabaceae. More than hundreds of plants have been reported in the Ethno-botanical literature of India for their anti-microbial activity as well as for treatment of many diseases and *Butea monosperma* (Lam.) Taub is one of them. The present investigation is focused on the anti-microbial activity of *Butea monosperma* leaves against both gram-positive (*Bacillus subtilis*, *Enterococcus faecalis*, *Lactobacillus acidophilus*, *Staphylococcus aureus* and *Streptococcus mutans*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) as well as fungi (*Candida albicans* and *Trichophyton rubrum*). Both excerpts (ethanol and methanol) show positive responses against all the selected microorganisms. GC-MS analysis is done to find out important compound which is anti-microbial. A total of 38 compounds are identified in GC-MS analysis. Out of 38 compounds, 8 compounds are identified as antimicrobial in nature such as Dodecane, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-], Hexadecanoic acid, methyl ester, Phytol, Palmidrol, Squalene, Tetracosane, Vitamin E and Lup-20(29)-en-3-one which justify, this plant possess significant anti-microbial property. GC-MS analysis reveals that majorly, this plant leaves also possess¹⁰ antioxidant compounds (Neophytadiene, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-], n-Hexadecanoic acid, Phytol, squalene, .alpha.-Tocospiro B, Tetracosane, .gamma.-Tocopherol, Ergost-5-en-3-ol, (3.beta.)- and STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-), 9 anti-cancerous compounds (Hexadecanoic acid, methyl ester, 9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, squalene, .gamma.-Tocopherol, Vitamin E, Ergost-5-en-3-ol, (3.beta.)-, STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-, .gamma.-Sitosterol and Lupeol), 8 anti-inflammatory compounds (Neophytadiene, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-], Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, Phytol, Palmidrol, .alpha.-Tocospiro B and Lupeol), 3 cardioprotective compounds (Neophytadiene, .alpha.-Tocospiro B and .gamma.-Tocopherol A) and 5 antidiabetic compounds (.alpha.-Tocospiro B, Vitamin E, STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-, .gamma.-Sitosterol and Lup-20(29)-en-3-one). This investigation suggests that this plant leaf has not only anti-microbial potential but also possesses other important compounds to fight against many deadly diseases. Thus, this plant has immense potential to make an individual place in pharmacological industries, especially as antibiotics.

Keywords: *Butea monosperma* (Lam.) Taub; Anti-microbial; GC-MS analysis

1. INTRODUCTION

In developing countries like India, 80% of people are depends on herbal medicine for their principal health care needs reported by WHO.¹ Different plant parts of Kingdom Plantae rich in the organic compound which is used as ethnic medicine from ancient times has mentioned in Ayurvedic, Chinese, and Unani medicine.^{1,2}

Ayurveda acts as a connecting link between plant and human health rights from human civilization. This plant is reported in Upanishads, Vedas, Susruta Samhita, Caraka Samhita, and in Astanga Hardaya and Astanga Sangraha.³ This tree also mention in 'Satpath Brahman', 'Matsya-purana', and 'Vau-Purana'. During Vedic times this tree is commonly known as 'Brahma-vriksha'. The Palash tree also specify in Mahabharata and Ramayana.⁴

Butea monosperma (Lam.) Taub is a reservoir of important chemical compounds which show significant physiological activities in the human body, so this plant can treat bacterial, fungal, and viral infections. Currently, Phyto-drugs are more popular than synthetic drugs because of their maximum benefits and fewer or no side effects.⁵

More than hundreds of plants have been reported in the Ethno-botanical literature of India for their anti-microbial activity as well as for treatment of many diseases and *Butea monosperma*(Lam.) Taub is one of them.⁶

Butea monosperma(Lam.) Taub is frequently branded as Palash (pal means leaf and sha means sacred, thus this plant represents; the leaf is pure and sacred), Dhak, and Muthuga which belong to the family Fabaceae.⁷ This medicinal plant is native to India, Pakistan, Sri

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Lanka, Bangladesh, Vietnam, Thailand, Indonesia, Laos, Thailand, and Cambodia.

The genus name *Butea* was given by Koenig in 1972 which represents a beautiful flower whereas the species name *monosperma* given by O. Kuntze in 1891 which is characteristic, each fruit contains only one seed in the apical end. During the spring season, the leaves are shed and trees are seen in full bloom with bright orange-coloured flowers (Fig. 1(a)) making it easily recognizable even to untrained eyes which gives the appearance of fire in the forest so this plant is also called the 'forest's flame'.

To authenticate the beauty of the Dhak plant flowers, government of Jharkhand make a State flower of Jharkhand. In India Palash positions second to *Schleichera oleosa* (Kusum) as the host of a lac insect.⁸ Palash tree grows up to 12-15 m high with irregular branches and crooked trunks (Fig. 1(b)). Leaves (Fig. 1(c)) are stipulate, trifoliate, alternate, petiolate and 10-15 cm long. Each leaflet is coriaceous, asymmetric, obtuse, glabrous, stipulate, reticulate, and petiolate. Leaf surface are pubescent and glutinous. The leaf margin is smooth and entire.

Flowers produced in racemes, terminal or axillary, bisexual with bract and bracteoles, pedicellate, and deciduous. Each flower (Fig. 1(d)) contains five petals, the outermost layer is called vexillum or standard. Two petals are united to form a cavity that is boat-shaped known as carina or keel, and two wings that look like the beak of a parrot. So, this plant is also called Kimsuka. The longest petal of flowers is the keel. This tree represents the God, Agnidev.⁹ The calyx is velvety, five-ridged, fleshy, gamosepalous, campanulate, and green. Corolla is scarlet, inside glossy and outside silvery tomentose. Stamens are diadelphous. The anther is bilobed, uniform, and basifixed. The ovary is linear, elongated, falcate, and sessile containing four-six ovules. Stigma is terminal, small and presents near to keel. Fruit (Fig. 1(e)) a pod, flat base, wing-like, oblong, the tip splitting around the apical seed, compressed, ovate seed^{4,10,11} (Fig. 1(f)). This plant well grows in both irrigated and dry land.

Almost all parts of the plant have medicinal importance as the roots show anti-parasitic activity¹², anti-fertility activity¹³; leaves show anti-microbial¹⁴, anti-filarial¹⁵, flower; free radical scavenging activity¹⁶, anti-microbial activity¹⁷, anti-diabetic activity¹⁸, hepatoprotective activity¹⁹, anti-cancer activity²⁰, bark; hepatoprotective²¹, anti-diarrheal²², anti-ulcer²³, fruit; anti-diabetic²⁴, seed; protease inhibitor activity²⁵, anti-fertility activity.²⁶

2. PHYTOCONSTITUENTS OF LEAVES

Leaves of Dhak plant show an abundance of glucoside, linoleic acid; antimicrobial²⁷, oleic acid; antimicrobial²⁷, lignoceric acid, and palmitic acid²⁸.

3. METHODOLOGY

3.1 Collection

Fresh leaves of Palash were collected from Namkum



Figure 1. Flowering plant (a), Non-flowering plant (b), Single leaf (c), Flowers (d), Fruits (e) and Seeds (f)

area (Ranchi district at lat 23°14'42''N, long 85°18'43''E) during March.

3.2 Identification

Fieldwork was carried out in the city of Ranchi for the identification of Palash plant leaf. The collected plant samples were tentatively identified with the help of keys and descriptions given in various floras²⁹ and monographs⁴ and later confirmed by matching with type

specimens present in the herbarium at the University Department of Botany, Ranchi University, Ranchi. The final authentication of this plant was done by the National Institute of Science Communication and Policy Research (authentication no.- NIScPR/RHMD/Consult/2022/4258-59-1), New Delhi.

3.3 Plant Extract Preparation

Collected leaves were washed properly with tap water and then wipe with a soft dry cloth to remove excess water and dust. These collected leaves were kept under shade dry conditions for about one month. Dry leaves were ground into fine powder. The cold extraction method was used for extract preparation.³⁰ Powder leaves are mixed into methanol at a 1:10 (w/v) ratio and then placed into a shaker-incubator for about 28°C, 80rpm for 72 hours for mixing. After that, mixture is filtered by using Whatman filter paper-1. Keep the filtrate under an incubator at 37°C, subjected to evaporation of methanol. A dry extract was prepared, called stock. Take 7mg of extract from this stock and mixed it with 1000µl of ethanol and methanol solvents. Both extracts were mixed properly by using a thermomixer at a temperature of 30°C, 1000rpm for 10 minutes and then centrifuged at 30°C, 1400rpm for 5 min. Removed the palate and the supernatant was transferred to a new centrifuge tube. This extract was used to test the antibiotic potential against selected microorganisms.

3.4 Anti-microbial Assay

Disc-diffusion method³¹ is used to test the anti-microbial commotion of the ethanol and methanol leaf excerpt of *Butea monosperma*. Bacteria are inoculated in nutrient agar media and fungus in SDS media on sterilized Petri plates. Sterilization was done by autoclave at a temperature of 120°C, 15 lbs pressure for 30-40 minutes. 100µl of microbial inoculum is uniformly spread in media by using a spreader (glass rod) and left to settle for about 1-2 minutes. After that 6mm of the disc is placed into the media and then different volumes (5 µl, 10 µl, and 15 µl) of concentration i.e., 7mg/1000µl of ethanol and methanol extract, were loaded into the disc. Petri plates were kept under two different sterilized (wiped by 70% ethanol) incubators, 37°C for bacteria and 28°C for fungus for 16-18 hours for maximum growth of microorganisms. The precinct of inhibition was calculated and compared with the standard. Tetracycline is used as the positive control for ethanolic extract and erythromycin for methanolic extract. Ethanol and methanol were used as a negative control.

3.5 Microorganism Used

Nine different microorganisms are accustomed to test the antimicrobial activity of leaf excerpts such as gram-positive bacteria; *Bacillus subtilis* (MCC 2511), *Enterococcus faecalis* (MCC 3040), *Lactobacillus acidophilus* (MCC LA), *Streptococcus mutans* (MCC SM), *Staphylococcus*

aureus (MCC 2408), gram-negative bacteria; *Pseudomonas aeruginosa* (MCC 3973), *Escherichia coli* (MCC 3099) and fungi; *Candida albicans* (MCC 1152) and *Trichophyton rubrum* (MCC 1850).

3.6 Calculations

A Triplicate is made of each experiment to calculate the mean value (mm) for standard calculation. The standard deviation is presented in ±form and the area of the inhibited zone is calculated in mm².

$$\text{Zone of inhibition (mm}^2\text{)} = \text{Area of inhibited zone } (\pi r_1^2) - \text{Area of disc } (\pi r_2^2)$$

The percentage of inhibition of Dhak plant leaves is calculated by using the formula:

$$\% \text{ inhibition} = \frac{\text{Zone of sample}}{\text{Zone of standard}} * 100$$

3.7 GC-MS Analysis

From stock, take 10mg of extract and mixed it with 1000µl of ethanol. Then this mixture is mixed by using a thermomixer and then a centrifuge. The palate was removed and the supernatant was transferred to the new centrifuge tube. Test samples were prepared was sent to Advanced Instrumentation Research Facility at Jawaharlal Nehru University, New Delhi. Shimadzu QP-2010 Plus with Thermal Desorption System TD 20 was used for this analysis. He (Helium) was used as carrier gas (flow rate of 16.3 mL/min and column flow rate of 1.21 mL/min) and temperature of column oven was set at 100°C. The total time for the GC-MS analysis test was 35 minutes. For calculating the phytoconstituents the acquired retention time (RT) and mass weight were compared with the spectra of the GC-MS database of NIST (National Institute of Standard and Technology) and Wiley library (online). An online literature survey was done by using the most popular sites such as Google Scholar, Pubchem, and Pub-med to investigate the bioactivity of phytoconstituents.

4. RESULTS

Day by day microorganisms modify themselves to reverse the activity of antibiotics, in such condition development of new antibiotics is essential, which have fewer or no side effects and maximum benefits to fight against such microorganisms. Nine different microorganisms were used in this experiment, which shows resistance such as *Bacillus subtilis* shows resistance against streptomycin³², *Enterococcus faecalis*; clindamycin, quinupristin-dalfopristin³³, *Lactobacillus acidophilus*; cephalosporins, vancomycin³⁴, *Streptococcus mutants*; fluoride resistance³⁵, *Staphylococcus aureus*; methicillin resistance³⁶, *Pseudomonas aeruginosa*; fosfomycin³⁷, *Escherichia coli*; beta lactum³⁸, *Candida albicans*; Flucanazole³⁹ and *Trichophyton rubrum*; azoles, amorolfine, terbinafine resistance⁴⁰.

This investigation shows, both the leaf excerpts of *Butea monosperma* inhibit the growth of all nine microorganisms which were selected for this experiment such as a- *Bacillus subtilis* (MCC 2511), b- *Enterococcus faecalis* (MCC 3040), c- *Lactobacillus acidophilus* (MCC LA), d- *Streptococcus mutans* (MCC SM), e- *Staphylococcus aureus* (MCC 2408), f- *Pseudomonas aeruginosa* (MCC 3973), g- *Escherichia coli* (MCC 3099) h- *Candida albicans* (MCC 1152) and i-*Trichophyton rubrum* (MCC 1850),

clearly shown in Fig. 2(a-i) and by ethanol excerpts and Fig. 3(a-i) by methanol excerpts of leaves. In ethanol excerpts maximum precinct of inhibition is conveyed against *Streptococcus mutans* i.e., 18.33 ± 0.33 (235.49) and the minimum against *Escherichia coli* i.e., 11.33 ± 0.33 (72.50) shown in Table 1. In methanol excerpts, the maximum precinct of inhibition is reported against *Candida albicans* i.e., 15 ± 0.57 (148.36) and the minimum against *Escherichia coli* i.e., 7.66 ± 0.33 (17.80) shown in Table 2.

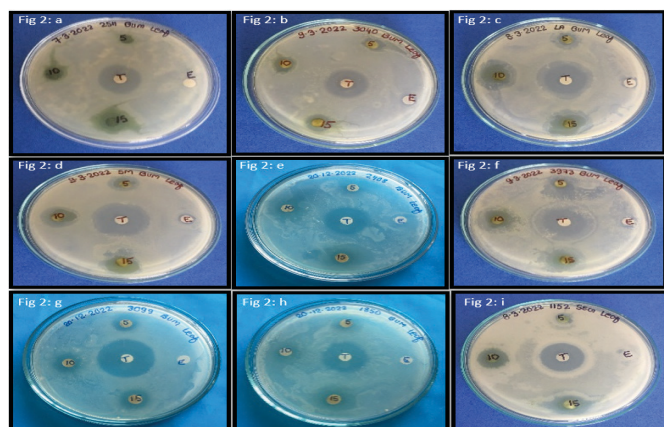


Figure 2. Anti-biotic potential of Dhak plant leaves excerpts (ethanol) against (a) *Bacillus subtilis* (MCC 2511), (b) *Enterococcus faecalis* (MCC 3040), (c) *Lactobacillus acidophilus* (MCC LA) (d) *Streptococcus mutans* (MCC SM), (e) *Staphylococcus aureus* (MCC 2408), (f) *Pseudomonas aeruginosa* (MCC 3973), (g) *Escherichia coli* (MCC 3099), (h) *Trichophyton rubrum* (MCC 1850) and (i) *Candida albicans* (MCC 1152).

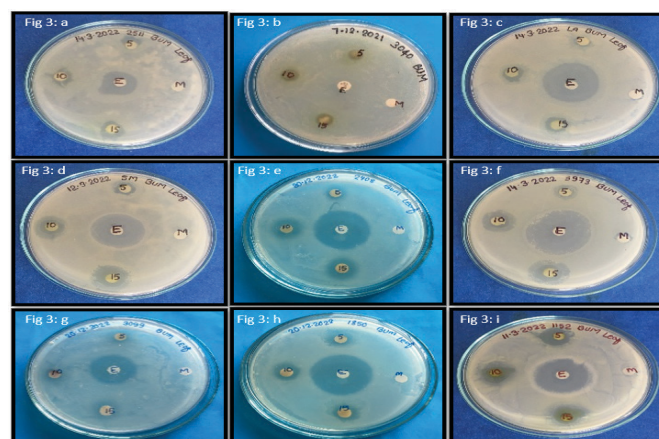


Figure 3. Anti-biotic potential of Dhak plant leaves excerpts (methanol) against (a) *Bacillus subtilis* (MCC 2511), (b) *Enterococcus faecalis* (MCC 3040), (c) *Lactobacillus acidophilus* (MCC LA) (d) *Streptococcus mutans* (MCC SM), (e) *Staphylococcus aureus* (MCC 2408), (f) *Pseudomonas aeruginosa* (MCC 3973), (g) *Escherichia coli* (MCC 3099), (h) *Trichophyton rubrum* (MCC 1850) and (i) *Candida albicans* (MCC 1152).

Table 1. Anti-microbial activity of ethanolic leaf excerpt of *Butea monosperma*

Microbial Strains	Zone of inhibition									
	5µl		10 µl		15 µl		T		E	
	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²
<i>Bacillus subtilis</i>	11.6 ± 0.33	77.36	13.66 ± 0.88	118.21	17.33 ± 0.88	207.49	21.33 ± 0.66	328.49	7 ± 0.33	10.20
<i>Enterococcus faecalis</i>	9.66 ± 0.33	44.99	11.66 ± 0.33	78.46	14.33 ± 0.33	132.93	20 ± 0.57	285.74	7.66 ± 0.33	17.80
<i>Lactobacillus acidophilus</i>	11 ± 0.57	66.72	14.66 ± 0.33	140.44	16 ± 0.57	172.7	25.33 ± 0.33	475.40	8.66 ± 0.66	30.61
<i>Streptococcus mutans</i>	13.66 ± 0.33	118.21	15.66 ± 0.33	164.24	18.33 ± 0.33	235.49	24.66 ± 0.88	449.11	8.33 ± 0.33	26.21
<i>Staphylococcus aureus</i>	7 ± 0.57	10.20	10.66 ± 0.66	60.94	15.33 ± 0.33	156.22	19 ± 0.57	255.12	6.33 ± 0.33	3.19
<i>Pseudomonas aeruginosa</i>	13.66 ± 0.66	118.21	15 ± 0.57	148.365	17.66 ± 0.33	216.56	24.66 ± 0.88	449.11	7.66 ± 0.33	17.80
<i>Escherichia coli</i>	9.33 ± 0.57	40.12	10.33 ± 0.57	55.56	12 ± 0.33	84.78	21.66 ± 0.88	340.02	-	-
<i>Trichophyton rubrum</i>	9.66 ± 0.33	44.99	12 ± 0.57	84.78	13.33 ± 0.66	111.22	19 ± 0.57	255.12	6.33 ± 0.33	3.19
<i>Candida albicans</i>	9.66 ± 0.66	44.99	14.66 ± 0.33	140.44	17 ± 0.57	198.60	25.66 ± 0.33	488.611	7 ± 0.57	10.205

Table 2. Anti-microbial activity of methanolic leaf excerpt of *Butea monosperma*

Microbial Strains	Zone of inhibition									
	5µl		10 µl		15 µl		Er		M	
	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²	Mean and SD (±)	mm ²
<i>Bacillus subtilis</i>	7± 0.57	10.20	8.66± 0.33	30.61	10.33±0.33	55.02	19± 0.57	255.12	6.33± 0.33	3.19
<i>Enterococcus faecalis</i>	6.66± 0.33	6.55	7.66± 0.33	17.80	8.33± 0.33	26.21	8.44± 0.33	27.65	7±0.33	10.26
<i>Lactobacillus acidophilus</i>	6.33± 0.33	3.19	7.66± 0.33	17.80	8.66± 0.33	30.61	21± 0.57	317.92	6.33± 0.33	3.19
<i>Streptococcus mutans</i>	7.66± 0.33	17.80	11± 0.57	66.72	13± 0.57	104.40	20.33±0.33	296.18	6.33± 0.33	3.19
<i>Staphylococcus aureus</i>	8.33± 0.33	26.21	10.33±0.88	55.50	11.66±0.88	77.36	23.33±0.33	399.00	6.33± 0.33	3.19
<i>Pseudomonas aeruginosa</i>	7.66± 0.33	17.80	11.33±0.33	72.50	14± 0.57	125.6	24± 0.57	423.9	-	-
<i>Escherichia coli</i>	-	-	6.66± 0.33	6.55	7.66± 0.33	17.80	18.33±0.33	235.49	-	-
<i>Trichophyton rubrum</i>	8.33± 0.33	26.25	9.66±0.57	45.09	10.83±0.66	63.86	19± 0.57	296.18	6.33± 0.33	3.19
<i>Candida albicans</i>	11.33±0.33	71.91	14± 0.57	125.6	15± 0.57	148.36	21± 0.57	317.92	6.33± 0.33	10.20

Anti-microbial study shows this plant leaf has immense potential to produce herbal antibiotics. The present study shows ethanol excerpts of leaves are more effective as compared to methanol excerpts. In ethanol excerpts, the maximum zone of inhibition is calculated against *Streptococcus mutans* and by methanol excerpts calculated against *Candida albicans*. Both the excerpts show minimum inhibitory effects against *Escherichia coli* (at higher volume, i.e., 15µl) seen in Fig. 4. The amount of drug loaded on the disc for both the excerpts are the same such as for the volume of 5µl were 35µg, 10 µl; 70 µg and 15 µl; 105 µg. The zone of inhibition by both excerpts was increased in a dose-dependent manner visualized in Figs 5 and 6.

The percentage of inhibition of ethanol and methanol excerpts of Dhak plant leaves is calculated. In ethanol excerpts of Dhak leaves, a maximum percentage of

inhibition, at a volume of 5µl was calculated against *Streptococcus mutans* and *Pseudomonas aeruginosa* i.e., 26.32%, at a volume of 10 µl was reported against *Streptococcus mutans* i.e., 36.57% and at a volume of 15µl is reported against *Bacillus subtilis* i.e., 63.16% respectively and minimum percentage of inhibition, at a volume of 5µl is reported against *Staphylococcus aureus* i.e., 3.99% and at volume 10µl and 15µl is reported against *Escherichia coli* i.e., 16.34% and 24.93% respectively, visualized in Fig. 7. In methanol excerpts, the maximum percentage of inhibition at a volume of 5µl, 10µl, and 15µl is reported against *Enterococcus faecalis* i.e., 23.68%, 64.37%, and 94.79% and minimum percentage of inhibition at a volume of 5µl, 10µl, and 15µl is reported against *Escherichia coli* i.e., 0%, 2.78% and 7.55%. visualized in Fig. 8.

GC-MS analysis is one of the important corridors

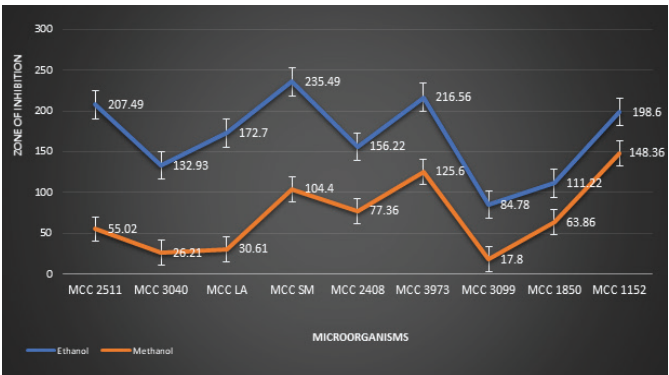


Figure 4. Comparison of the inhibitory zone of ethanolic and methanolic leaf excerpts of *Butea monosperma*

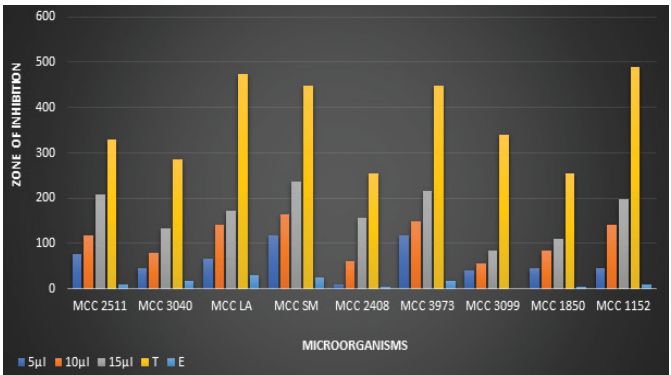


Figure 5. Dose-dependent activity of Ethanol excerpts against microbial pathogens

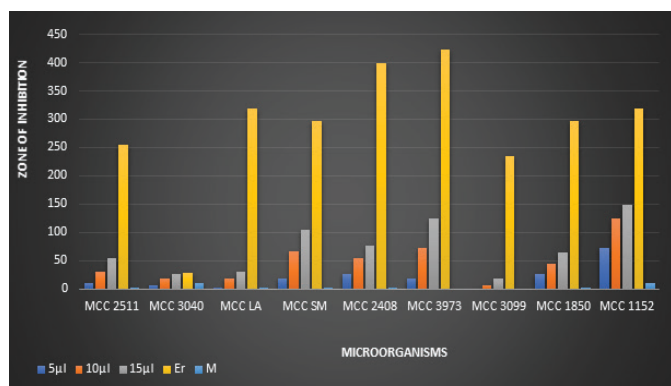


Figure 6. Dose-dependent activity of methanol extracts against microbial pathogens

for the identification of the important volatile compound from the test sample. By this instrumental technique (GC-MS) analysis, 38 compounds are pointed out by different peaks of chromatograms. Out of these 38 compounds, mome inositol shows the uppermost peak area% of 53.00% at a retaining time of 12.294 minutes, and the minimum showed by dodecane with a peak area% of 0.11 at 10.115 minutes, retention time. Retention time, percentage of peak area, chemical compound, molecular weight, nature, and molecular formula are mentioned in Table 3. The bioactivity of 20 chemical compounds is identified, mentioned in Table 4, which are effective against many diseases.

5. CONCLUSION

The present investigation provides scientific validation thus these plant leaves are a potent source of antibiotics against all selected microorganisms. The maximum percentage of inhibition is found against *Enterococcus faecalis* (methanol). Ethanol extracts of Dhak plant

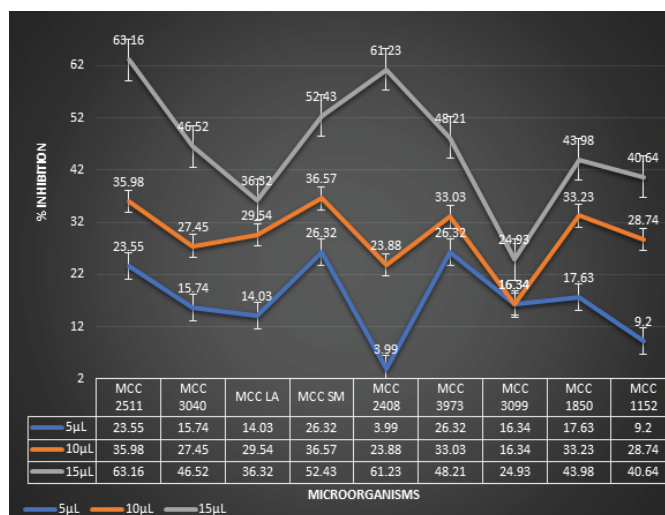


Figure 7. % inhibition by ethanol extracts of Dhak plant leaves against microbial pathogen

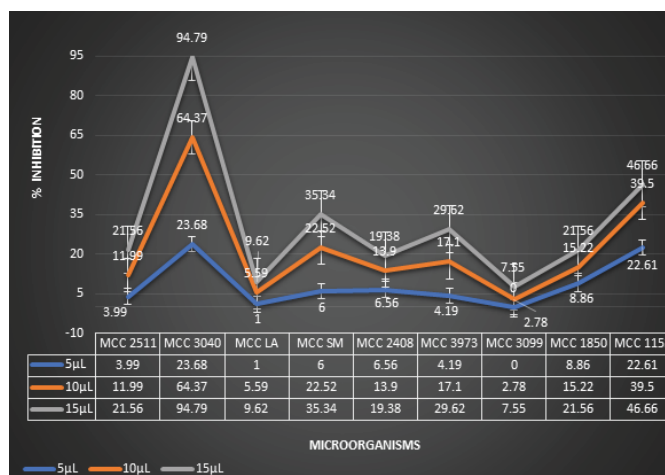


Figure 8. %inhibition by methanol extracts of Dhak plant leaves against microbial pathogen

Table 3. Retention time, percentage of peak area, chemical compound, molecular weight, nature, the structure of chromatograms, molecular formula

S. No.	R.T.	Peak area %	Chemical compounds	Molecular Weight (g/mol)	Nature	Mf
1	10.115	0.11	Dodecane	170.33	Clear colourless liquid.	C ₁₂ H ₂₆
2	10.617	0.14	MEGASTIGMATRIENONE 4	190.28	Nor – isoprenoids (βcarotene)	C ₁₃ H ₁₈ O
3	10.974	0.29	4,4,5,8-Tetramethylchroman-2-ol	206.28	Alkaloid	C ₁₃ H ₁₈ O ₂
4	12.294	53.00	MOME INOSITOL			
5	12.757	7.69	Neophytadiene	278.5	Diterpene	C ₂₀ H ₃₈
6	13.208	2.73	2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-]		Phytol	
7	13.575	0.22	Dibutyl phthalate	278.34	Phthalate ester	C ₁₆ H ₂₂ O ₄
8	13.685	0.31	Hexadecanoic acid, methyl ester	270.5	Fatty acid methyl ester	C ₁₇ H ₃₄ O ₂
9	14.140	0.32	n-Hexadecanoic acid	256.42	Saturated long-chain fatty acid	C ₁₆ H ₃₂ O ₂

10	15.316	0.06	Carbonic acid, ethyl undec-10-enyl ester	242.35		$C_{14}H_{26}O_3$
11	15.376	0.28	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER	292.5	Linoleic acid, methyl este	$C_{19}H_{32}O_2$
12	14.479	4.70	Phytol	296.5	Acyclic diterpene alcohol and a constituent of chlorophyll.	$C_{20}H_{40}O$
13	16.211	0.09	Bicyclo[3.1.1]heptan-3-one, 2,6,6-trimethyl-, (1.alpha.,2.alp			
14	17.130	0.10	14-.BETA.-H-PREGNA			
15	17.303	0.17	Palmidrol	299.5	ethanolamide of palmitic (hexadecanoic) acid	$C_{18}H_{37}NO_2$
16	17.371	0.31	2-Pyrrolidinone, 1-[2-(4-piperidiny)ethyl]-	196.29		$C_{11}H_{20}N_{20}$
17	18.767	0.49	HEXATRIACONTANE	507	Alkane	$C_{36}H_{74}$
18	18.987	0.08	4-Methoxy-2-methyl-1-butanol, TMS derivative	190.35		$C_9H_{22}O_2Si$
19	19.069	1.96	1,2-BENZENE DICARBOXYLIC ACID	166.13	Benzene dicarboxylic acid	$C_8H_6O_4$
20	19.555	1.10	3,7,11-trimethyldodeca-2,6,10-trien-1-yl palmitate	460.8		$C_{31}H_{56}O_2$
21	20.289	0.63	1,54-DIBROMOTETRAPENTACONTANE	917.2		$C_{54}H_{108}Br_2$
22	20.421	0.49	2,6,10,14-HEXADECATETRAEN, 1-ACETOXY-3,7,11,15			
23	20.986	0.48	3,7,11-trimethyldodeca-2,6,10-trien-1-yl palmitate	460.8		$C_{31}H_{56}O_2$
24	21.120	1.17	Squalene	410.7	Triterpene	$C_{30}H_{50}$
25	21.384	0.17	.alpha.-Tocospiro B			
26	21.544	0.34	.alpha.-Tocospiro B			
27	21.758	0.19	Tetracosane	338.7	Straight-chain alkane	$C_{24}H_{50}$
28	21.949	0.16	3,7,11,15-TETRAMETHYL-2,6,10,14-HEXADECATETR			
29	23.238	0.25	.gamma.-Tocopherol	416.7	Tocopherol	$C_{28}H_{48}O_2$
30	23.769	0.46	(3S,8S,9S,10R,13R,14S,17R)-17-((2R,5R)-5-Ethyl-6-methyl	38.062	Phytosterols	$C_{29}H_{50}O$
31	24.078	3.27	Vitamin E	430.7	Fat-soluble	$C_{29}H_{50}O_2$
32	25.498	0.86	Ergost-5-en-3-ol, (3.beta.)-	400.7	Steroid derivative	$C_{28}H_{48}O$
33	25.845	3.03	STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-	412.7	Steroid derivative	$C_{29}H_{48}O$
34	26.791	5.13	.gamma.-Sitosterol	414.7	Phytosterols	$C_{29}H_{50}O$
35	27.528	1.22	.beta.-Amyrin	426.7	Triterpenes	$C_{30}H_{50}O$
36	27.813	1.15	Lup-20(29)-en-3-one	424.7	Triterpenoid	$C_{30}H_{48}O$
37	28.180	0.36	4,4-Dinorlupan-4-one-28-carboxylic acid, 3.beta.-methyl-, m			
38	28.360	6.52	Lupeol	426.7	Pentacyclic triterpenoid	$C_{30}H_{50}O$

Table 4. Bioactivity of a chemical compound

S.No.	Chemical compound	Biological activity
1	Dodecane	Anti-bacterial ⁴¹
4	MOME INOSITOL	Anti-cirrhotic, lipotropic, anti-alopecic, anti-neuropathic cholesterolytic, and have sweetening properties ^{42,43}
5	Neophytadiene	Important for treatment of headaches, anti-rheumatism, cardioprotective, anti-oxidant, anti-inflammatory and used for cure skin disease ⁴⁴
8	Hexadecanoic acid, methyl ester	Anti-inflammatory, antiarthritic, anti-coronary, hypocholesterolemic, anti-cancer, antiacne, hepatoprotective, antihistaminic, antieczemic, alpha-reductase inhibitor, antiandrogenic, nematocide ⁴⁵
9	n-Hexadecanoic acid	Anti-inflammatory, anti-androgenic, anti-oxidant ⁴⁶
11	9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER	Anti-plasmodial, urine acidifier, and urinary acidulant, arachidonic acid inhibitor also inhibits the production of uric acid, anti-inflammatory, anti-cancerous ⁴⁷
12	Phytol	Anti-microbial, anti-tuberculosis, anti-convulsant, antinociceptive, anti-inflammatory, antioxidant ⁴⁸
15	Palmidrol	Non-Steroidal, analgesics, anti-Inflammatory, antihypertensive, neuroprotective, antiviral, anticonvulsant, anti-oxidative ⁴⁹
24	Squalene	Anti-cancerous, anti-bacterial, anti-oxidant, immunostimulant UV ray protection ⁵⁰
25	.alpha.-Tocospiro B	Anti-oxidant, anti-diabetic, anti-inflammatory, cardioprotective, hypocholesterolemic ⁵¹
26	.alpha.-Tocospiro B	Anti-oxidant, anti-diabetic, anti-inflammatory, cardioprotective, hypocholesterolemic ⁵¹
27	Tetracosane	Ant-microbial, anti-oxidant ⁵²
29	.gamma.-Tocopherol	Anti-cancerous, anti-tumour, anti-oxidant, cardioprotective, hypocholesterolemic ^{53,54}
31	Vitamin E	Major known anti-ageing and anti-dermatic compounds. It also possesses anti-diabetic, anti-tumour, anti-cancerous, hypocholesterolemia, anti-bronchitis, and anti-coronary ^{55,56}
32	Ergost-5-en-3-ol, (3.beta.)-	Anti-cancerous, Liver disease, Atherosclerosis, Jaundice, Antioxidant ⁵⁷
33	STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-	Hypolipidemic agent, anti-cancerous, antioxidant, anti-diabetic, exhibit antihepatotoxic and is the major source of synthetic progesterone, hypocholesterolemic ⁵⁷
34	.gamma.-Sitosterol	Anti-cancer, anti-diabetic ⁵⁸
35	.beta.-Amyrin	Antitumor ⁵⁹
36	Lup-20(29)-en-3-one	Have melanogenesis activity, anti-diabetic ⁶⁰ , anti-bacterial, anti-HIV ⁶¹
38	Lupeol	Anti-Inflammatory, Anti-cancerous, anti-arthritis, anti-diabetes ⁶²

leaves show more than 50% of inhibition against *Bacillus subtilis*, *Streptococcus mutans* and *Staphylococcus aureus* at a volume of 15µl. In methanolic excerpts, more than 50% of inhibition is found against *Enterococcus faecal* is at a volume of 10µl and 15µl. Out of 38 compounds

which are identified in GC-MS analysis, 8 compounds are identified as anti-microbial in nature such as Dodecane, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-], Hexadecanoic acid, methyl ester, Phytol, Palmidrol, Squalene, Tetracosane, Vitamin E and Lup-20(29)-en-

3-one which justify, this plant have significant anti-microbial property. This plant leaves also possesses 9 anti-cancerous compounds such as Hexadecanoic acid, methyl ester, 9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, squalene, .gamma.-Tocopherol, Vitamin E, Ergost-5-en-3-ol, (3.beta.)-, STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-, .gamma.-Sitosterol and Lupeol show this plant is very effective against deadly cancer disease which ranks second for worldwide death.⁶³ Leaves of dhak are also rich in the anti-oxidant compound where 10 compounds are antioxidants such as Neophytadiene, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-, n-Hexadecanoic acid, Phytol, squalene, .alpha.-Tocospino B, Tetracosane, .gamma.-Tocopherol, Ergost-5-en-3-ol, (3.beta.)- and STIGMASTA-5,22-DIEN-3-OL, (3.BETA.,22E)-. 8 compounds are anti-inflammatory in nature such as Neophytadiene, 2-HEXADECEN-1-OL, 3,7,11,15-TETRAMETHYL-, [R-, Hexadecanoic acid, methyl ester, n-Hexadecanoic acid, 9,12,15-OCTADECATRIENOIC ACID, METHYL ESTER, Phytol, Palmidrol, .alpha.-Tocospino B and Lupeol. Leaves are also rich in 3 cardioprotective compounds such as Neophytadiene, .alpha.-Tocospino B and .gamma.-Tocopherol. 5 antidiabetic compounds such as alpha. -Tocospino B, Vitamin E, STIGMASTA-5,22-DIEN-3-OL, (3. BETA.,22E) -, .gamma. -Sitosterol and Lup-20(29)-en-3-one are also justifying that these plant leaves have the potential for the production of herbal medicines. This investigation suggests that this plant leaf has not only anti-microbial potential but also possesses other important compounds to fight against many deadly diseases. Thus, this plant has immense potential to make an individual place in pharmacological industries.

6. DISCUSSION

Hot leaf excerpts of the Dhak plant show antibiotic potential against *P. aeruginosa*, *S. aureus*, *K. pneumoniae*, *E. coli*, and *C. freundii*.¹⁴ Methanol excerpts of Dhak plant leaves are effective against *S. typhi*, *B. megaterium*, *P. Vulgaris*, *E. coli*, *S. typhi* A and B and *B.cerus* as well as Fungi, *Rhizopus stolonifera*, and *A. niger*.⁶⁴ Water and ethanol of Dhak plant bark are significant against *B. cereus*, *P. aeruginosa*, and *E. coli*. Maximum inhibition is reported against *B. cereus* (water) and *E. coli* (ethanol) i.e., 26 mm. Gentamicin is used as a standard drug.⁶⁵ Dhak plant shows significant anti-microbial activity against *S. aureus*, *B. subtilis* and *E. coli*.⁶⁶ GC-MS analysis of Dhak plant leaves is not previously reported. These plant leaves are rich in important phytochemicals such as flavonoids, phenol, tannin, saponin and alkaloids.^{67,68} Alkaloids,⁶⁹ tannins⁷⁰ and flavanoids⁷¹ possess antimicrobial properties. Phenols are effective against *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*.⁷² Saponins are effective against *E. coli*.⁷³ *Butea monosperma* is one of the known ethnomedicinal plant which help to fight against many diseases.⁷⁴ If the present investigation has collaborated with previous work, the effectiveness of Dhak plant leaves reported previously against *E. coli* and *B. subtilis*, *Pseudomonas aeruginosa* and *Staphylococcus* species justify this plant as a potent source of antibiotics.

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CONTRIBUTORS

Ms. Surabhi Ambastha completed her M.Sc. (Botany) in the year 2019 from Ranchi University, Ranchi. Her area of interest is ethnomedicinal. Presently, she is a Research Scholar.

In the present study, she carried out the work in the laboratory and prepared the figures, tabulation, calculation, and clinical significance of the research under the supervision of the corresponding author.

Dr. Amit Patnaik is Ph.D. (Molecular biology) from Ranchi University, Ranchi in the year 2016. His area of interest is Cancer Biology, Bioinstrument and Infection disease and he is presently working as an Assistant Professor (Biotechnology), University Department of Botany, Ranchi University, Ranchi. He worked on concept development, data analysis, and article amendment involved in the present research work.

Dr. Vinay Oraon is Ph.D. (Microbiology) from Barkatullah University, Bhopal in the year 2010. His area of interest is Bacteriology, Molecular Biology, Bioinformatics and phylogenetics and he is presently working as an Assistant Professor, University Department of Botany (Biotechnology), Ranchi University, Ranchi. He carried out the clinical significance and verification of the herbal medicinal plant involved in the present research work

Dr. Latika Sharan received her PhD (Algal Flora-Green Algae) from Ranchi University. She is co-author of a book i.e., Environmental Science, and Ethics, and co-author of a chapter in a book i.e., Ethnobotany, cultivation, and utilization of plants. She has experience in the field of Microbiology and wrote papers on it. She is presently working as an Associate Professor at the University Department of Botany, Ranchi University, Ranchi. She guided and supervised the work in the laboratory and prepared the finding of the research. She reviewed and inferences with the manuscript.