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BIOACTIVE COMPOUND IN *CURCUMA CAESIA* (ROXB.) FROM BASTAR AND ITS SPECTRAL ANALYSIS BY HPLC, UV-VISIBLE, FT-IR, NMR, AND ESI-MS

Dhananjay Pandey ^{*1} and A. K. Gupta ²

School of Studies in Biotechnology ¹, Bastar Vishwavidyalaya, Dharampura, Jagdalpur - 494001, Chhattisgarh, India.

Microbiology Research Laboratory ², School of Studies in Life Science, Pt. Ravishankar Shukla University, Raipur - 492010, Chhattisgarh, India.

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Correspondence to Author:

Dr. Dhananjay Pandey

Guest Faculty (Biotechnology),
DST-INSPIRE Fellow,
School of Studies in Biotechnology,
Bastar Vishwavidyalaya, Dharampura,
Jagdalpur - 494001, Chhattisgarh,
India.

E-mail: pandey.dhananjay333@gmail.com

ABSTRACT: The increasing failures of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbes have led to the screening of several medicinal plants for their potential antimicrobial activity. Thus, the present endeavour deals with the exploration of antibacterial efficacy of *Curcuma caesia* (Roxb.) (Family Zingiberaceae) against bacterial cultures procured from IMTECH, Chandigarh as a screen for potential candidates for the development of natural antibiotics. The agar well diffusion assay was performed to screen antibacterial activity, and ZOI was recorded. The results were analyzed using one way ANOVA with DMRT. A broth macro-dilution assay was used to determine (MIC) and (MBC). Finally, the purified fraction was chemically characterized by spectral analysis. The antibacterial activity profile revealed that the methanolic root extract exhibited broad-spectrum antibacterial potentiality. The extract exhibiting the highest antibacterial activity was purified by column chromatography and further with HPLC, and TLC analyzed the band pattern. The bio-efficacy of the purified fraction was assessed against pathogenic bacteria, and the MIC and MBC were determined. The maximum zone of inhibition was recorded against *B. cereus* (MTCC 430) and *K. pneumoniae* (MTCC 3384). The purified fraction from a crude extract of *C. caesia* (Roxb.) indicates that the molecular weight of the purified compound is 368, the molecular formula is C₂₁H₂₀O₆. Its chemical characteristics and structural properties were found to be similar to that of 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione. However, this is the first report of the presence of curcumin in *C. caesia* (Roxb.) from Bastar demonstrating antibacterial property.

INTRODUCTION: The therapeutic power of herbs had been recognized since the creation of the universe, and botanic medicine is one of the oldest practiced professions by humanity ¹.

In many parts of India, herbal medicines are documented to be effective in the alleviation of several diseases such as malaria, dysentery, jaundice, diabetes, cough, fever, snakebite, miscarriages and gonorrhoea ². There are several reports on the antimicrobial activity of herbal extracts in different parts of the world ^{3, 4, 5}. Therefore, in light of the present context, an effort to further explore the medicinal or natural products towards improving health care delivery deserves attention.

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Curcuma is a large genus belonging to the family Zingiberaceae, which comprises over 70 species of rhizomatous herbs ⁶. *Curcuma caesia* (Roxb.) popularly known as Kali Haldi (Black turmeric) is an uncommon endemic and perennial herb with bluish-black rhizome. It is native to North-East, and Central India, relatively unexplored medicinal plant valued all over Asia for its therapeutic efficacy. It flourishes well in moist deciduous forest areas with rich humid and clayey soils.

In India, it is found in Chhattisgarh, Madhya Pradesh, Odisha, Uttar Pradesh and West Bengal ⁷. It is also sparsely found in Papi Hills of East Godavari, West Godavari and Khammam district of Andhra Pradesh. *C. caesia* (Roxb.) possess immense ethnomedicinal importance and has been used by various tribal communities in the amelioration of several dreadful diseases such as asthma, leucoderma, tumors, piles, and bronchitis. It also possesses anti-oxidant, anti-tumor, anti-asthmatic, anti-inflammatory, hepatoprotective, blood purifier, stomachic and carminative properties ⁸.

Chhattisgarh is identified as an 'Herbal State' due to its rich repository of medicinal and aromatic plants in many tribal districts including Bastar. The tribes of Bastar region are traditionally dependant on plants for curing their ailments since long. Despite the rich abundance of medicinal flora in Bastar, the region is relatively less explored concerning the antimicrobial properties of medicinal plants. Microbiologists, natural product chemists, and ethnobotanists are in search of novel bioactive compounds from medicinal plants for

curing several infectious diseases ⁹. Thus, in light of vast potentiality of medicinal plants as sources of antimicrobial compounds, an effort was made to assess the antimicrobial efficacy of *Curcuma caesia* (Roxb.) from the Bastar region of Chhattisgarh.

MATERIALS AND METHODS: Bastar district (19.1071°N, 81.9535°E) is located in the southern part of Chhattisgarh and has an area of 4029.98 km². It is surrounded by Bijapur, Dantewada, Kondagaon, Narayanpur and Sukma districts of the state. Bastar, the land of tribals and natural resources, is also surrounded by dense forests, hilly mountains, natural caves, waterfalls, and streams. Jagdalpur is both district and divisional headquarter of Bastar district. The city lies on the southern bank of river Indravati with an average elevation of 562 meters. It has a total forest area of 292130 ha which is more than 19% of the total land area of the district.

Selection and Identification of Medicinal Plants: Medicinal plant viz., *Curcuma caesia* (Roxb.) (Family: Zingiberaceae), was selected for study from Bastar region based on the ethnomedicinal importance and therapeutic usage by the tribal community as the source of food, medicine and cosmetics ¹⁰. Traditionally wild plants consisting of several bioactive phytochemicals are used as a source of herbal preparations possessing therapeutic properties ^{11, 12}. The *Curcuma caesia* (Roxb.) was collected and identified at the Department of Agronomy and Horticulture, SGCARS, Jagdalpur, Chhattisgarh, India **Fig. 1**.

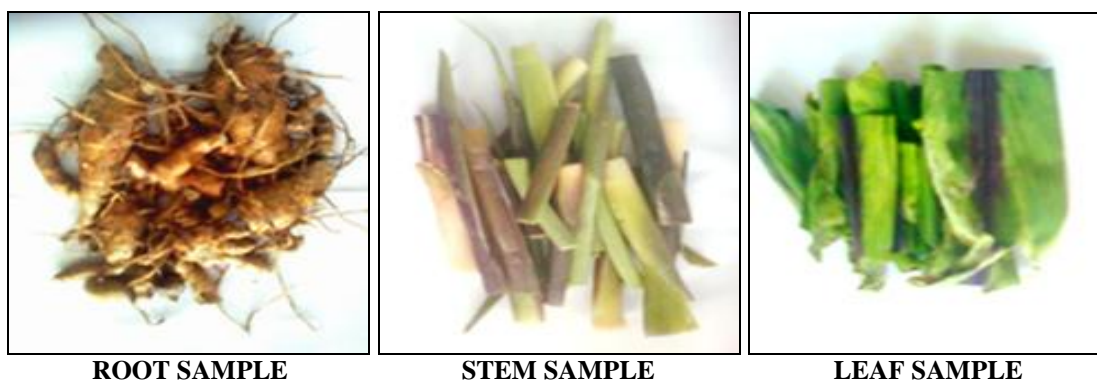


FIG. 1: DIFFERENT PARTS OF CURCUMA CAESIA (ROXB.)

Extraction Method: The extraction of phytochemicals was done through hot extraction. In Soxhlet apparatus, powdered material was placed

in a thimble of filter paper. The powdered plant material was extracted sequentially in four different solvents viz., chloroform, acetone, methanol and

aqueous. 15 g powdered material was extracted in 150 ml of chloroform, acetone, methanol and in aqueous according to their increasing polarity index in the Soxhlet apparatus (Tempo) for 8 - 10 h at a temperature not exceeding the boiling point of the respective solvents. The extracted material was dried to the residue. The sample was dissolved in 50% dimethyl sulphoxide to prepare a 10% stock solution (w/v) and stored in a refrigerator at 4 °C in small sterile glass tubes.

Microorganism for Antimicrobial Activity:

Antimicrobial activity was assessed against *B. cereus* (MTCC 430), *B. subtilis* (MTCC 441), *S. aureus* (MTCC 96), *S. epidermidis* (MTCC 435), *E. coli* (MTCC 1687), *K. pneumoniae* (MTCC 3384), *P. aeruginosa* (MTCC 741) and *P. vulgaris* (MTCC 744) procured from IMTECH, Chandigarh, India.

Preparation of Bacterial Inoculums: The test organisms were maintained on nutrient agar slants. An overnight grown bacterial culture was used for inoculum preparation. One loop full of overnight growth from each bacterial culture was inoculated in 25 ml nutrient broth and incubated at 37 °C for 24 h in the incubator. The inoculum size of each bacterial strain was standardized by adjusting the optical density of the culture broth by adding saline suspension to a turbidity corresponding to 0.08-0.13 at 620 nm using a spectrophotometer which was equivalent to 10^8 cfu/ml¹³.

Assessment of Antibacterial Activity: The antibacterial activity of crude extracts was determined by the agar-well diffusion method¹⁴. 200 µl of the standardized cell suspension was spread on Muller Hinton Agar (Hi-media) plate using a sterile swab and air dried to remove the surface moisture. 6 mm diameter wells were bored into the agar plate using a sterile cork borer. The crude extract was introduced into the well at a concentration of 2 mg / 20 µl. The plates were allowed to stand at room temperature for about 1 h as a period of pre-incubation diffusion to minimize the effect of variation in time between the application of different solutions and later the plates were incubated at 37 °C for 24 h. Controls were also set up in parallel, and the effects were compared with penicillin and streptomycin at a concentration of 10 µg / 20 µl.

The plates were observed for the zone of inhibition after 24 h. The experiment was conducted in triplicates, and the values are expressed as Mean ± SE. All the results were analyzed statistically wherever required by one way-ANOVA using SPSS version 16.0 software.

Column Chromatography: The column chromatography was performed in a glass column (50 × 2 cm). The column was erected straight on a stand. In the setting up of the column, the lower part of the glass column was packed with glass wool. The slurry was prepared by mixing 20 g of silica gel (60-120 mesh) in 100 ml chloroform. The slurry was poured carefully into the column taking care that no air traps in and were allowed to settle for 2 h. The column was tapped from outside to eliminate the air bubbles if any. The top of the glass column was open to allow free flow of solvent into a conical flask. The set up was considered to be in order when the solvent drained freely without carrying either silica gel or glass wool. At the end of the packing process, the tap was closed. The column was washed with 150 ml eluent and allowed to stabilize for 24 h. The flow rate of the solvent was kept at 2 ml/min. 5 ml of crude sample was loaded in the column and elution of the extract was done with solvent systems of gradually increasing polarity using chloroform, acetone, and methanol. The following ratios of solvent combinations were sequentially used in the elution process viz., chloroform: acetone 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100. Acetone: methanol was used in a ratio of 100:0, 80:20, 60:40, 40:60, 20:80 and 0:100 sequentially. The eluted fractions were collected in aliquots of 5 ml in test tubes for further analysis.

Thin Layer Chromatography (TLC): The thin-layer plate (20 × 20 cm) was prepared by spreading aqueous slurry of the silica gel G. (8 g in 100 ml distilled water) on a clean surface of the glass to obtain a thickness of 0.25 mm. The plate so prepared was activated in an oven at 110 - 120 °C for 30 min before sample loading. The crude and purified methanol root extracts of *C. caesia* (Roxb.) applied as a spot using the capillary tube, 3 cm above the edge of the plate. The plate was air dried for evaporating sample solvent. The plate was placed in the solvent system, chloroform: methanol [95:5 v/v].

After completion of the run-up to two-thirds of the length of the plate, the plate was examined in the ultra-violet chamber, photographed and the spots were identified, and their R_f values were determined.

Minimum Inhibitory Concentration (MIC): The broth macrodilution sensitivity test was performed following CLSI to determine minimum inhibitory concentration (MIC) of the plant extract¹⁵. The test was performed in clean and sterile glass test tubes (12 × 75 mm) using Muller Hinton Broth (Hi-media). The plant extract was taken in the first tube, and serial two-fold dilutions of the extract were prepared in successive test tubes and mixed thoroughly to give final concentrations ranging from 1-0.0156 mg/ml for bacterial cultures. 0.5 ml of bacterial inoculum was added to all the tubes.

Appropriate solvent blanks and standard antibiotics were also incubated as negative and positive controls respectively. Tests were carried out in duplicates. The cultured tubes were sealed with parafilm and incubated at 37 °C for 24 h for bacteria. The MIC of the sample was detected following the addition of 50 µl of 0.5% TTC (2, 3, 5 Triphenyl Tetrazolium Chloride, Hi-media) in all the test tubes and incubated at 37 °C for 30 min.

Microbial growth was determined by observing the change of color of TTC in the tubes (Pinkish-red formazan when there is growth and clear solution when there is no growth) without shaking them¹⁶. The lowest concentration of the plant extract that

inhibited the growth of bacteria was taken as MIC. MIC value < 0.5 mg/ml was defined as potential strong activity.

Minimum Bactericidal Concentration (MBC): Minimum Bactericidal Concentration (MBC) was determined by spot inoculation method. A sample was taken from each test tubes along with the control tube and spotted on Muller Hinton agar (Hi-media) plates with the help of sterile cotton swabs. Plates were incubated at 37 °C for 24 h for bacteria. All the experiments were performed in duplicates. MBC was defined as the lowest concentration of the plant extractable to kill most of the bacteria with 99.9% of effectiveness as compared with control growth.

Chemical Characterization of the Bioactive Compound: The bioactive fraction was chemically characterized by spectral analysis using HPLC, UV-Visible spectroscopy, FT-IR, NMR, and ESI-MS.

RESULTS: The antibacterial activity of root of *C. caesia* (Roxb.) revealed the maximum zone of inhibition against *B. cereus* (15.06 ± 0.06 mm) and minimum by *B. subtilis*. Amongst Gram-negative bacteria, *K. pneumoniae* showed the highest inhibition (11.80 ± 0.11 mm) whereas, *P. aeruginosa* was found to be resistant against all the extracts tested. However, leaf and stem extracts exhibited comparatively less antimicrobial activity against all the microbes tested **Fig. 2 - 3**.

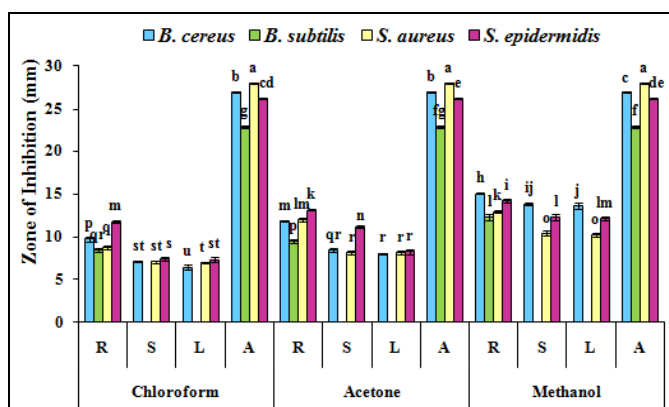


FIG. 2: ANTIBACTERIAL ACTIVITY OF ROOT (R), STEM (S) AND LEAF (L) EXTRACTS OF C. CAESIA (ROXB.) AGAINST GRAM-POSITIVE BACTERIA vis-à-vis STREPTOMYCIN (A) (ANOVA Summary: $F_{47, 96} = 3544.00$, $p < 0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p < 0.001$) (Based on Duncan's multiple-range test)

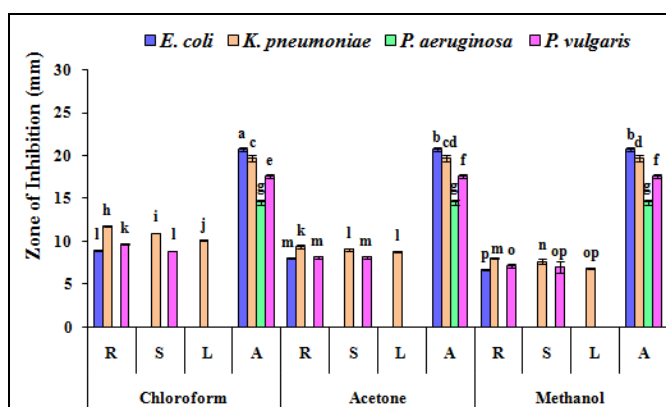


FIG. 3: ANTIBACTERIAL ACTIVITY OF ROOT (R), STEM (S) AND LEAF (L) EXTRACTS OF C. CAESIA (ROXB.) AGAINST GRAM-NEGATIVE BACTERIA vis-à-vis STREPTOMYCIN (A) (ANOVA Summary: $F_{47, 96} = 4553.00$, $p < 0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p < 0.001$) (Based on Duncan's multiple-range test)

Active crude methanol root extract exhibiting high antibacterial activity was purified by column chromatography for further characterization. The 37th and 38th fraction gave the highest antibacterial activity against *B. cereus* and *K. pneumoniae*. They were pooled and subjected to TLC along with crude methanol root extract. The crude extract showed four prominent spots with the R_f values of 0.96, 0.95, 0.93 and 0.91. The pooled fractions showed single spot with the R_f value of 0.96 **Fig. 4 - 5** and **Table 1**. The pooled fraction was further analyzed for its purity by HPLC, and the purified product

(98.59%) was obtained with the retention time of 3.637 min **Fig. 6**.

TABLE 1: R_f VALUE OF CRUDE METHANOL EXTRACT AND PURIFIED FRACTION OF *C. CAESIA* (ROXB.) BY TLC

Spots	R _f Values	Colour of Spot
a. Purified pooled fraction	0.96	Yellow
b. Crude methanol extract		
Spot 1	0.96	Yellow
Spot 2	0.95	Purple
Spot 3	0.93	Light Pink
Spot 4	0.91	Dark Blue

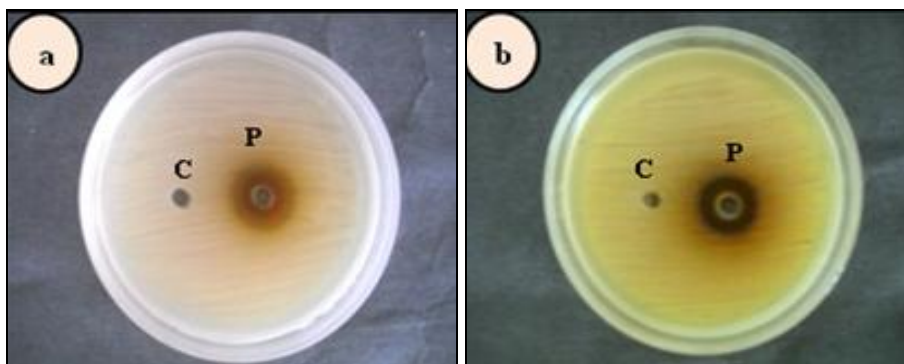


FIG. 4: ANTIBACTERIAL ACTIVITY OF THE POOLED 37th AND 38th FRACTIONS OF *C. CAESIA* (ROXB.) AGAINST (A) *B. CEREUS* (B) *K. PNEUMONIAE* (C- CONTROL AND P- PURIFIED FRACTION)

The MIC and MBC of the crude extract and purified fraction were evaluated for pathogenic bacteria. The crude methanol root extract of *C. caesia* (Roxb.) showed the MIC and MBC value 0.25 mg/ml for *B. cereus* and *S. epidermidis*; 0.5 mg/ml for *B. subtilis* and *S. aureus*.

K. pneumoniae; 0.5 mg/ml for *E. coli* and *P. vulgaris* and 1 mg/ml for *P. aeruginosa* **Table 2**.

However, the corresponding purified fraction showed the value of 0.0625 mg/ml for *B. cereus*; 0.125 mg/ml for *S. epidermidis* and 0.25 mg/ml for *B. subtilis* and *S. aureus*. In case of Gram-negative bacteria the crude extract exhibited MIC & MBC value 0.5 mg/ml for *K. pneumoniae* and 1 mg/ml for both *E. coli* and *P. vulgaris* whereas, the value >1 mg/ml was recorded for *P. aeruginosa*. The purified fraction showed the value 0.25 mg/ml for

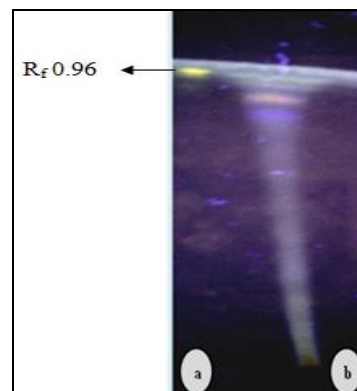


FIG. 5: TLC PROFILE OF METHANOL ROOT EXTRACT OF *C. CAESIA* (ROXB.) (A) PURIFIED POOLED FRACTIONS (B) CRUDE METHANOL ROOT EXTRACT

TABLE 2: MIC AND MBC OF CRUDE EXTRACT AND PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

S. no.	Bacterial Cultures	Crude extract (mg/ml)	Purified fraction (mg/ml)	Positive control (µg/ml)
1	<i>B. cereus</i>	0.25	0.0625	0.312
2	<i>B. subtilis</i>	0.50	0.25	0.625
3	<i>S. aureus</i>	0.50	0.25	0.156
4	<i>S. epidermidis</i>	0.25	0.125	0.156
5	<i>E. coli</i>	1	0.50	0.625
6	<i>K. pneumoniae</i>	0.50	0.25	1.25
7	<i>P. aeruginosa</i>	>1	1	2.50
8	<i>P. vulgaris</i>	1	0.50	1.25

Chemical Characterization of Purified Fraction from *C. caesia* (Roxb.): The bioactive purified methanol root fraction from *C. caesia* (Roxb.) was characterized by spectral studies *i.e.*, UV-Visible spectroscopy, FT-IR, NMR, and ESI-MS.

UV-Visible Spectroscopy: The purified fraction from root methanol extract of *C. caesia* (Roxb.) analyzed by UV-Visible spectroscopy indicated the presence of a carbonyl group and aromatic ring with maximum absorption at λ_{max} (methanol) at 420 nm with an absorbance of 1.624 **Fig. 7**.

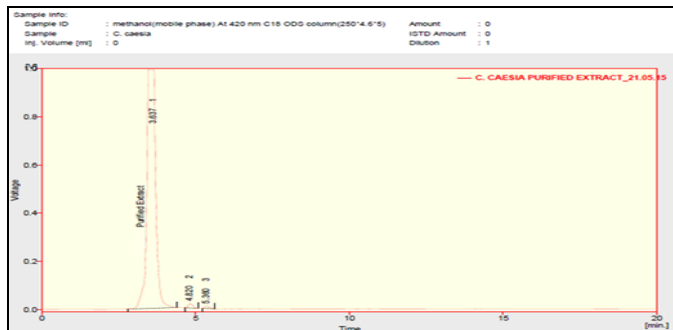


FIG. 6: HPLC CHROMATOGRAM OF THE PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

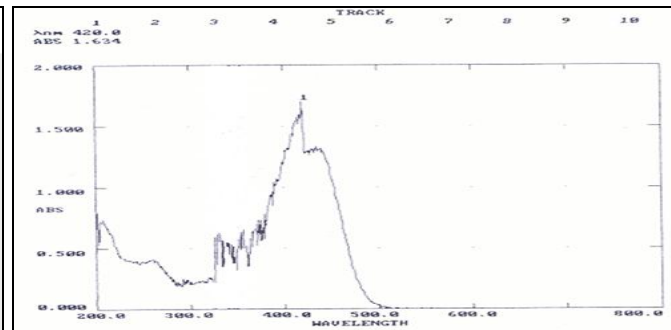


FIG. 7: ULTRAVIOLET VISIBLE SPECTRUM OF THE PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

Fourier Transmission Infra-red (FT-IR) Spectroscopy: An FT-IR spectrum of pure methanol root fraction at 500-4000 cm^{-1} is presented in **Fig. 8** using KBr pellet technique that showed peaks at different wavenumber range. The characteristic peaks were observed at 3393.90 (O-H stretch, H-bonded), 1596.16 (C-C stretch in the ring), 1495.86 (C-C stretch in the ring), 1761.08 (C=O stretch), 1114.90, 1069.57 (C-O stretch) and 991.45, 917.19 (=C-H bend) respectively.

percent, frequency (Freq) and coupling constant (J) in hertz. The spectrum of the compound revealed the presence of different types of neighboring H atoms and their splitting **Fig. 9**.

Nuclear Magnetic Resonance (NMR) Spectroscopy: ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra of the compound in deuterated water (D_2O) were recorded with 300 MHz Bruker DR X.

The spectrum showed the presence of different δ values *viz.*, $\delta=7.592$ (d, Int=11.455, Freq=3034.98, J=12.82); $\delta=7.552$ (d, Int=12.319, Freq=3019.11, J=15.86); $\delta=7.221$ (t, Int=15.318, Freq=2886.66, J=132.44); $\delta=7.216$ (t, Int=15.340, Freq=2884.83, J=1.83); $\delta=7.118$ (s, Int=7.773, Freq=2845.77, J=39.06); $\delta=7.114$ (s, Int=6.910, Freq=2843.94, J=1.83); $\delta=7.098$ (s, Int=9.206, Freq=2837.83, J=6.10); $\delta=7.094$ (s, Int=8.377, Freq=2836.00, J=1.83); $\delta=6.827$ (t, Int=17.781, Freq=2729.19, J=106.81); $\delta=6.807$ (t, Int=16.897, Freq=2721.26, J=7.93); $\delta=6.651$ (d, Int=11.671, Freq=2659.00, J=62.25); $\delta=6.613$ (d, Int=11.537, Freq=2643.74, J=15.25); $\delta=5.964$ (s, Int=9.202, Freq=2384.34, J=259.39) and $\delta=3.909$ (m, Int=100.00, Freq = 1562.812, J=821.53).

^1H NMR (D₂O exchangeable) δ : The ^1H NMR spectra of the purified fraction of *C. caesia* (Roxb.) was recorded and the corresponding chemical shift values (δ) are expressed in ppm, intensity (Int) in

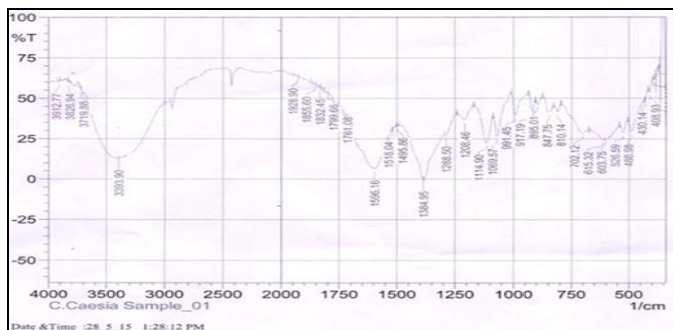


FIG. 8: INFRARED (IR) SPECTRUM OF THE PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

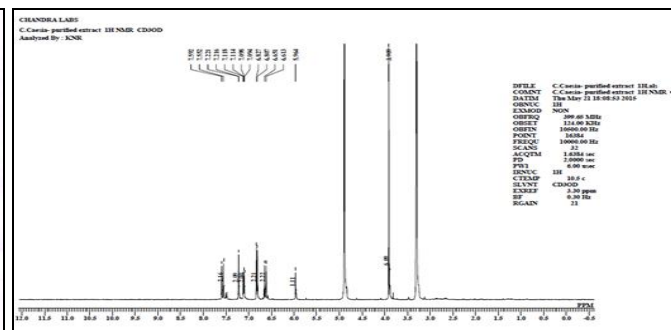


FIG. 9: ^1H NMR SPECTRUM OF THE PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

^{13}C NMR (D₂O exchangeable) δ : ^{13}C NMR showed different peaks at 56.434 (-CH₃ group), 116.566-116.879 (C=C of aromatic ring), 122.238-124.132 (C-C of aromatic ring), 142.145 (C=C of aliphatic chain), 149.423-150.476 (C-O of aromatic ring) and 184.766 (>C=O or carbonyl of aliphatic ring) respectively. The spectrum showed the presence of different C atoms in the compound **Fig. 10**.

Electro Spray Ionization Mass Spectrometry (ESI-MS): The molecular weight of the purified

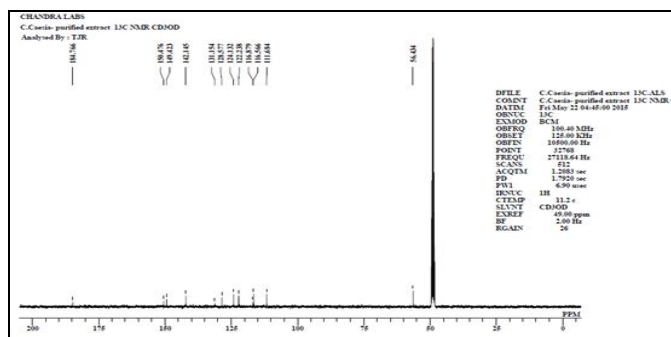


FIG. 10: ^{13}C NMR SPECTRUM OF THE PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

DISCUSSION: The antibacterial activity of *C. caesia* (Roxb.) revealed that in case of gram-positive bacteria the maximum zone of inhibition and activity index was exhibited by *B. cereus* and minimum by *B. subtilis*. Amongst Gram-negative bacteria, *K. pneumoniae* showed the highest inhibition whereas, *P. aeruginosa* was found to be resistant against all the extracts tested. The leaf and stem extracts exhibited comparatively less antimicrobial activity against all the microbes tested. The results revealed that methanol root extracts were found to be more potent than other solvent extracts. Similar findings were reported in *Azadirachta indica* (Meliaceae), *Allium cepa* (Liliaceae) and *Aloe vera* (Liliaceae); in *Phyllanthus niruri* and *Emilia sonchifolia* (Asteraceae)^{17, 18, 19}. However, no antimicrobial activity was recorded for aqueous extracts against all the organisms tested. The results suggested that organic solvents were more suitable for the extraction of the active principles in *C. caesia* (Roxb.) as compared from aqueous solvent for antimicrobial potential. Several authors have observed similar findings in various plants²⁰⁻³⁰.

The active crude methanol root extract of *C. caesia* (Roxb.) was purified by silica gel column

chromatography using different solvent gradients, and a total of 48 fractions were obtained. The highest antibacterial activity was recorded in 37th and 38th fraction against gram-positive (*B. cereus*) and gram-negative (*K. pneumoniae*) bacteria by agar well diffusion method. Both the fractions were pooled and subjected to TLC along with crude methanol root extract. The crude methanol root extract of *C. caesia* (Roxb.) showed four prominent spots with the R_f values 0.96, 0.95, 0.93 and 0.91 respectively. The pooled fraction showed a single spot with the R_f value of 0.96. Similar values were also reported in *Curcuma longa* with similar solvent system³¹. The pooled fraction was further analyzed for its purity by HPLC, and the purified product (98.59%) was obtained which showed the retention time of 3.637 min.

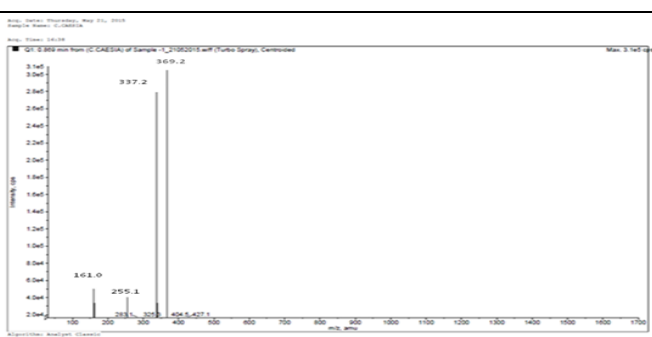


FIG. 11: MASS SPECTRUM OF THE PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* (ROXB.)

The MIC and MBC of the methanol crude root extract and purified pooled fraction were evaluated against both gram-positive and gram-negative bacteria. The crude methanol root extract of *C. caesia* (Roxb.) showed higher MIC and MBC value (0.25-0.5 mg/ml) for *B. cereus* (MTCC 430), *S. epidermidis* (MTCC 435), *B. subtilis* (MTCC 441) and *S. aureus* (MTCC 96). However, the corresponding purified fraction showed much

chromatography using different solvent gradients, and a total of 48 fractions were obtained. The highest antibacterial activity was recorded in 37th and 38th fraction against gram-positive (*B. cereus*) and gram-negative (*K. pneumoniae*) bacteria by agar well diffusion method. Both the fractions were pooled and subjected to TLC along with crude methanol root extract. The crude methanol root extract of *C. caesia* (Roxb.) showed four prominent spots with the R_f values 0.96, 0.95, 0.93 and 0.91 respectively. The pooled fraction showed a single spot with the R_f value of 0.96. Similar values were also reported in *Curcuma longa* with similar solvent system³¹. The pooled fraction was further analyzed for its purity by HPLC, and the purified product (98.59%) was obtained which showed the retention time of 3.637 min.

lower value (0.0625-0.25 mg/ml) for *B. cereus*, *S. epidermidis*, *B. subtilis* and *S. aureus*.

Similarly, in case of gram-negative bacteria, the crude extract exhibited higher MIC & MBC value (0.5 - > 1 mg/ml) for *K. pneumoniae* (MTCC 3384), *E. coli* (MTCC 1687), *P. vulgaris* (MTCC 744) and *P. aeruginosa* (MTCC 741). The respective purified fraction exhibited much lower value (0.25-1 mg/ml). The Researchers have reported lower MIC & MBC value of pure compound *vis-a-vis* crude extract in case of *Combretum micranthum* and *Guiera senegalensis*³².

The UV-Visible spectroscopy, Fourier Transmission Infra-red spectroscopy, Nuclear Magnetic Resonance spectroscopy, and Electron Spray Ionization Mass spectrometry of the purified compound from crude methanol root extract of *C. caesia* (Roxb.) indicates that molecular weight of the purified compound is 368, the molecular formula is C₂₁H₂₀O₆ with different functional groups. Its chemical characteristics and structural properties were found to be similar to that of 1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione. Several reports of curcumin conferring antibacterial properties are available in the literature in different species of *Curcuma*^{33, 34}. However, this is the first report of the presence of curcumin in *C. caesia* (Roxb.) from Bastar demonstrating antibacterial property. Varying content of curcumin in different species of the genus *Curcuma* was reported³⁵. It would also be rewarding to analyze the curcumin content in *C. caesia* (Roxb.) from different ecotypes.

CONCLUSION: The present study was carried out to assess the antibacterial activity in the root, stem and leaf extracts of *Curcuma caesia* (Roxb.) against gram-positive and gram-negative bacteria. The results were promising and showed significant broad-spectrum antibacterial activity which was evident from the lowered MIC and MBC values of the purified fraction as compared to crude extracts. The purified fraction was further characterized using HPLC, UV-Visible spectroscopy, FT-IR, NMR (1H & 13C) & ESI-MS. The result revealed that purified fraction of methanol root extract of *C. caesia* (Roxb.) was chemically characterized as curcumin (C₂₁H₂₀O) possessing antibacterial

activity. This is the first report of the presence of curcumin in *C. caesia* (Roxb.) from Bastar demonstrating antibacterial property.

However, it would also be rewarding to analyze the curcumin content in *C. caesia* (Roxb.) from different ecotypes.

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