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ANTIBACTERIAL EFFICACY OF *CURCUMA CAESIA* FROM BASTAR DISTRICT OF CHHATTISGARH, INDIA

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ABSTRACT: Bio-efficacy of plants and their derivatives have been reemphasized in recent times. The present investigation was carried out to study the antibacterial efficacy of *Curcuma caesia* (family: Zingiberaceae) commonly known as kali haldi. The root, stem and leaf of *Curcuma caesia* were extracted successively with polar (aqueous, methanol), dipolar (acetone) and non-polar (chloroform) solvents, and their physical characteristics were explored which revealed the presence of more phytocompounds in case of root followed by stem and leaf. The extracts were assessed for their potential antibacterial activity against gram positive and gram negative bacteria *viz*., *Bacillus cerus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Klebsiella pneumoneae*. The gram positive bacteria were found to be more sensitive than gram negative bacteria. The inhibition of both gram positive and gram negative bacteria by the extracts indicates the presence of broad spectrum antibacterial potentiality of the plant. The root extracts of the plant were found to be more effective in inhibiting the bacterial growth as compared to stem and leaf. The root extracts of the plant showed inhibition against all gram positive and gram negative bacteria except *Pseudomonas aeruginosa*. The highest activity index was recorded in root methanol and chloroform extracts in case of *Bacillus cerus* and *Klebsiella pneumoneae* respectively. The results were promising and justified the use of *Curcuma caesia* root by tribal community of Bastar and traditional healers in combating several bacterial diseases.

INTRODUCTION: Medicinal plants are one of the emerging and best sources for the discovery and development of novel bioactive compounds for combating several bacterial diseases [1]. The plant based herbal therapy is widely explored in the traditional system of medicine and their curative potentials are well documented [2].

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The medicinal properties of plants are due to their antioxidant, antimicrobial, antipyretic, anti-inflamatory and antitumour activity of the phytochemicals present in them [3].

The use of plant extracts for the treatments of several bacterial diseases have become popular because the effective life span of antibiotic is limited and over prescription as well as misuse of antibiotics is causing antimicrobial resistance [4]. Many reports have documented the effective activity of traditional herbs against microorganisms, so plants are one of the bedrocks for the discovery of modern medicines [5].

Several types of antibiotics are available, but a large number of factors such as, drug toxicity, low potency, emergence of resistant bacterial strains, high cost of new generation antibiotics with limited effective span have resulted in increased death rate [6]. However, over the past few decades the health benefits are under threat as many commonly used antibiotics and their extensive use against bacterial diseases has led to the emergence of multidrug resistance [7]. The bacterial species have developed the genetic potentiality to acquire and transmit resistance [8]. Herbal medicines are in great demand in the developed as well as developing countries due to their wide medicinal and biological applications.

So the identification of bioactive compounds in plants, their isolation, purification and characterization of active ingredients in crude extracts by various analytical methods is greatly significant. Thus, the documentation of the plants phytochemicals to treat and prevent infectious bacterial diseases has attracted the attention of scientist's worldwide [9].

Curcuma species have a great importance for its medicinal value. *Curcuma caesia* is a member of family Zingiberaceae and popularly known as kali haldi. It has been used by various tribal communities in curing several ailments since traditional times. *C. caesia* is a perennial herb with bluish-black rhizome native to North-East and Central India. In India it is found in Chhattisgarh, Madhya Pradesh, Uttar Pradesh, Orissa and West Bengal. Black Turmeric is also sparsely found in Papi Hills of East Godavari, West Godavari and Khammam Districts of Andhra Pradesh. The rhizomes of kali haldi have a high economic importance because of its putative medicinal properties. The rhizomes are used in the treatment of smooth muscle relaxant activity, leprosy, dirrhoea, wound, asthma, haemorrhoids, cancer, epilepsy, fever, vomiting, menstrual disorder, antihelmentic, aphrodisiac, inflammation and gonorrhoeal discharges [10, 11]. The antimicrobial activities of this plant have not yet been explored. In this context present study was carried out to screen the antibacterial efficacy of *Curcuma caesia* against both gram positive and gram negative bacteria *viz*., *Bacillus cerus, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa* and *Klebsiella pneumoneae.*

MATERIALS AND METHODS:

Selection of medicinal plant: Plant of *Curcuma caesia* was selected based on its traditional usage by the tribal community of Bastar district of Chhattisgarh in healing several diseases and its ethno-medicinal significance as herbal drug. Apparently healthy and disease free plants were selected for antibacterial screening.

Collection of the sample: The fresh and healthy root, stem and leaves of *Curcuma caesia* were collected and identified at Department of Horticulture, Shahid Gundadhoor College of Agriculture and Research Station, Kumhrawand, Jagdalpur from Bastar district, Chhattisgarh, India. The plant samples were washed under running tap water to remove debris and shade dried for about three weeks to attain a constant weight. The dried samples were mechanically grinded by using a mortar and pestle and finally powdered by laboratory grinder machine and stored in separate air tight bottles till use (**Fig. 1**).

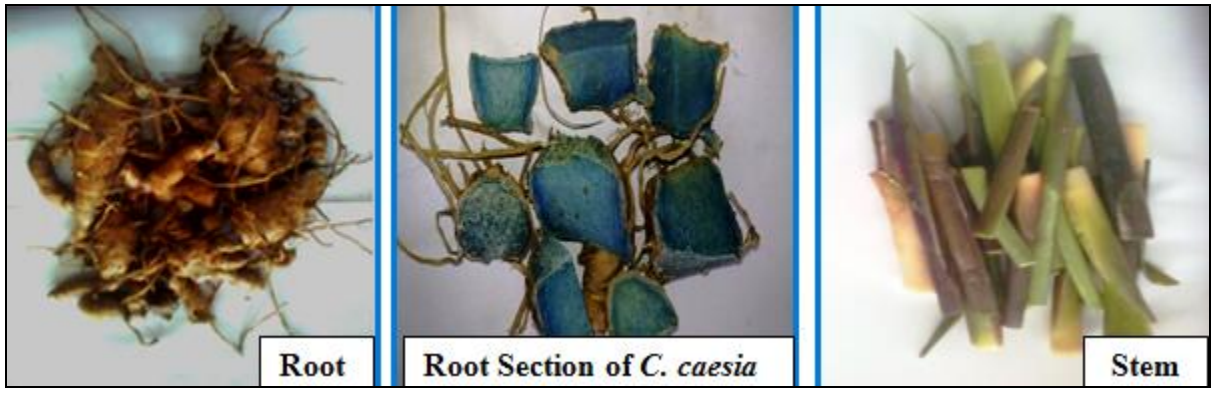

FIG. 1: SAMPLE COLLECTION OF DIFFERENT PARTS OF *CURCUMA CAESIA*

Extraction procedure: 15g powdered material was extracted with 150 ml of different solvents according to their increasing polarity successively for 8-10 hours in the Soxhlet apparatus at a temperature not exceeding the boiling point of the respective solvents. After extraction excess solvent was removed by distillation and the concentrated extracts so obtained were further dried in incubator at 40^{o}C. The percentage yield and other physical properties were recorded. The residual extracts after drying were dissolved in 50% DMSO and stored in refrigerator at 4^{o}C in small and sterile glass tubes.

Microorganisms used for the test: The present study was carried out with the bacterial strains procured from IMTECH, Chandigarh, India. The bacterial strains used for antibacterial screening were *Bacillus cereus* (MTCC-430), *Bacillus subtilis* (MTCC-441), *Staphylococcus aureus* (MTCC-96), *Staphylococcus epidermidis* (MTCC-435), *Escherichia coli* (MTCC-1687), *Proteus vulgaris* (MTCC-744), *Pseudomonas aeruginosa* (MTCC-741) and *Klebsiella Pneumoniae* (MTCC-3384). The bacterial strains were maintained on nutrient agar slants, sub cultured regularly and stored at 4^{o}C for further use.

Inoculum preparation: One loop full of overnight grown bacterial culture was inoculated in 25 ml nutrient broth at 37^{o}C on a rotary shaker incubator for 16-18 h. The inoculum size of each bacterial strains were standardized by adjusting the optical density of the culture broth to a turbidity corresponding to 0.08 at 620 nm using a spectrophotometer which is equivalent to 10^{8} cfu/ml [12].

Assessment of antibacterial activity: The antibacterial activity of the crude extracts was determined by the agar-well diffusion method[13]. 200 µl of the standardized cell suspension were spread on Muller Hinton Agar (Hi-media) plate using a sterile swab and air dried to remove the surface moisture. Wells were then bored into the agar using a sterile 6 mm diameter cork borer. The crude extract was introduced into the well at a concentration of 2mg/20µl, 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^{o}C for 24 h. Controls were also set up in parallel and the effects were compared with penicillin and streptomycin at a concentration of 10µg/20µl. The plates were observed for the zone of inhibition after 24 h. The experiment was conducted in triplicates and the results are expressed as mean ± SE.

RESULT AND DISCUSSION: The various parts of *Curcuma caesia viz.*, root, stem and leaf were extracted successively using four different solvents based on their polarity index as chloroform, acetone, methanol and aqueous for assessment of their antibacterial efficacy as the antibacterial phytochemical compounds of the plant origin may occur mostly in root, stem and leaf of the plants [14]. The physical properties of the root, stem and leaf extracts of *Curcuma caesia* were studied and the result revealed that the root extracts were almost sticky to viscous in nature with brown to yellowish in color and the percentage yield was found to be highest in root extracts in case of polar aqueous solvent (6.33%) followed by methanol (4.80%), acetone (1.93%) and chloroform (1.40%).

The highest percentage yield in aqueous solvent might be due to the fact that water is a universal solvent and extracts most of the compounds [15]. The stem extracts were greenish to brown in color and leaf extracts were almost waxy to viscous in nature with dark green to brown in color and the percentage yield of the stem and leaf extracts were found to be maximum in case of polar organic solvents (5.06%) and non-polar solvents (4.73%) respectively (**Table 1**). The above study clearly illustrates the presence of varied phytochemical in different parts of the plants and their affinity towards different solvents according to their polarity index.

TABLE 1: PHYSICAL CHARACTERISTICS OF THE EXTRACT OF *CURCUMA CAESIA* IN DIFFERENT SOLVENTS

Characteristics	% Yield	Color	Consistency
ROOT			
Chloroform	1.40	Dark Brown	Sticky
Acetone	1.93	Brown	Viscous pasty
Methanol	4.80	Reddish Brown	Less viscous
Aqueous	6.33	Yellowish	Semi solid
STEM			
Chloroform	4.53	Dark greenish	Sticky
Acetone	1.60	Light brown	Viscous
Methanol	5.06	Brownish	Semi solid
Aqueous	1.13	Brown	Less viscous
LEAF			
Chloroform	4.73	Dark green	waxy
Acetone	0.40	Brownish	Viscous pasty
Methanol	2.46	Greenish brown	Viscous
Aqueous	3.80	Dark brown	Highly viscous

The efficacy in crude extract of root, stem and leaf of *Curcuma caesia* in four different solvents based on their polarity as polar (aqueous, methanol), dipolar (acetone) and non-polar (chloroform) were assessed against both gram positive and gram negative bacteria *viz*. *Bacillus cerus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Proteus vulgaris Pseudomonas aeruginosa* and *Klebsiella pneumoneae*. The study revealed that the root extracts were found to be more effective to these bacteria followed by stem and leaf.

The antibacterial activity in case of gram positive bacteria was found to be maximum in methanolic root extracts followed by acetone and chloroform. The methanolic root extract exhibited significant zone of inhibition against all the four gram positive bacteria with maximum against *Bacillus cerus* (15.06±0.06) followed by *Staphylococcus epidermidis* (14.20±0.20), *Staphylococcus aureus* (12.93±0.17), and *Bacillus subtilis* (12.33±0.33). The stem and leaf extract showed activity against *Bacillus cerus* followed by *Staphylococcus epidermidis* and *Staphylococcus aureus*, whereas no significant activity was observed in case of their aqueous extract. These results clearly indicate that organic solvents were more suitable for the extraction of the active principles responsible for antibacterial activity [16, 17].

The decline in activity of aqueous extract might be due to the excessive heating of the aqueous soluble active constituents during the extraction process which often affect biologically active substances such as flavonoids, essential oils and other heterogeneous phytoconstituents present in the extract [18, 19, 20]. The phytochemical analysis of *Curcuma caesia* showed the presence of alkaloids, flavonoids, tannins, polyphenolics, terpenoids, phytosterols, resins and saponin which might be responsible for the antimicrobial activity of plant extracts [21]. The potential sensitivity of the extracts against gram positive bacteria and the zone of inhibition were recorded and are presented in **Table 2 and Fig. 2**.

TABLE 2: ANTIBACTERIAL ACTIVITY OF *CURCUMA CAESIA* AGAINST GRAM POSITIVE BACTERIA (ZONE OF INHIBITION IN MM, MEAN ± SE)

Bacterial Species	*B. cerus*	*B. subtilis*	*S. aureus*	*S. epidermidis*
ROOT				
Chloroform	09.73±0.26	08.46±0.24	08.80±0.20	11.80±0.11
Acetone	11.86±0.13	09.46±0.24	12.00±0.23	13.13±0.06
Methanol	15.06±0.06	12.33±0.33	12.93±0.17	14.20±0.20
Aqueous	ND	ND	ND	ND
Penicillin	11.86±0.13	10.33±0.24	26.40±0.40	29.33±0.33
Streptomycin	26.93±0.06	22.86±0.13	27.93±0.06	26.20±0.11
STEM				
Chloroform	07.13±0.06	ND	07.06±0.17	07.46±0.24
Acetone	8.46±0.24	ND	08.20±0.20	11.20±0.11
Methanol	13.86±0.13	ND	10.40±0.30	12.33±0.33
Aqueous	ND	ND	ND	ND
Penicillin	11.86±0.13	10.33±0.24	26.40±0.40	29.33±0.33
Streptomycin	26.93±0.06	22.86±0.13	27.93±0.06	26.20±0.11
LEAF				
Chloroform	06.46±0.29	ND	06.93±0.06	07.33±0.33
Acetone	08.06±0.06	ND	08.20±0.20	08.26±0.26
Methanol	13.66±0.33	ND	10.26±0.26	12.13±0.24
Aqueous	ND	ND	ND	ND
Penicillin	11.86±0.13	10.33±0.24	26.40±0.40	29.33±0.33
Streptomycin	26.93±0.06	22.86±0.13	27.93±0.06	26.20±0.11

Standard antibiotics: Penicillin and Streptomycin; ND- Not detected

The antibacterial activity in case of gram negative bacteria was found to be comparatively less than that of gram positive bacteria against all the extracts tested. The chloroform extract of root exhibited maximum zone of inhibition against *Klebsiella pneumoneae* (11.80±0.11) followed by *Proteus vulgaris* (09.73±0.06), *Escherichia coli* (09.00±0.00) and no significant activity was observed in case of *Pseudomonas aeruginosa*. However, the stem and leaf extracts showed activity against *Klebsiella pneumoneae* and *Proteus vulgaris*.

Amongst gram negative bacteria, *Klebsiella pneumoneae* showed the highest inhibition whereas *Pseudomonas aeruginosa* were found to be resistant against all the extracts tested (**Table 3 and Fig. 3**). In the present investigation the gram positive bacteria were found to be more susceptible as compared to gram negative bacteria against the plant extracts tested. The higher resistance of gram-negative bacteria to plant extracts is due to thick murein layer in their outer membrane, which prevents the entry of inhibitory substances into the cell and have outer phospholipid membrane carrying the structural lipopolysaccharides components, this makes the cell wall impermeable to antimicrobial substances whereas, gram positive bacteria have single layered cell wall with peptidoglycan constituting the outer layer, which is not an effective permeability barrier [22, 23].

The activity index was calculated to express the relationship between zones of inhibition of the extracts with the standard antibiotics [24]. Among the root, stem and leaf extracts of *Curcuma caesia*, the highest activity index of 0.55 was recorded in methanol extract of root in gram positive bacteria as *B. cerus* whereas, activity index of 0.59 was observed for gram negative bacteria as *K. pneumoneae* against streptomycin (**Table 4**).

Higher activity index (>0.5) in the crude extract indicates potential antibacterial activity in the plant. The findings of present study offers a scientific validation for the usage of *Curcuma caesia* root by the tribal community of Bastar as their food, medicine, cosmetics and traditional healers in curing different diseases.

TABLE 3: ANTIBACTERIAL ACTIVITY OF *CURCUMA CAESIA* AGAINST GRAM NEGATIVE BACTERIA (ZONE OF INHIBITION IN MM, MEAN ± SE)

Bacterial Species	*E. coli*	*P. vulgaris*	*P. aeruginosa*	*K. pneumoneae*
ROOT				
Chloroform	09.00±0.00	09.73±0.06	ND	11.80±0.11
Acetone	08.13±0.13	08.20±0.20	ND	9.46±0.24
Methanol	06.80±0.11	07.20±0.20	ND	8.06±0.06
Aqueous	ND	ND	ND	ND
Penicillin	09.93±0.29	ND	ND	ND
Streptomycin	20.80±0.20	17.66±0.24	14.60±0.23	19.73±0.37
STEM				
Chloroform	ND	8.93±0.06	ND	11.00±0.00
Acetone	ND	08.20±0.11	ND	09.13±0.13
Methanol	ND	07.06±0.66	ND	07.73±0.26
Aqueous	ND	ND	ND	ND
Penicillin	09.93±0.29	ND	ND	ND
Streptomycin	20.80±0.20	17.66±0.24	14.60±0.23	19.73±0.37
LEAF				
Chloroform	ND	ND	ND	10.20±0.11
Acetone	ND	ND	ND	08.86±0.13
Methanol	ND	ND	ND	06.93±0.06
Aqueous	ND	ND	ND	ND
Penicillin	09.93±0.29	ND	ND	ND
Streptomycin	20.80±0.20	17.66±0.24	14.60±0.23	19.73±0.37

Standard antibiotics: Penicillin and Streptomycin; ND- Not detected

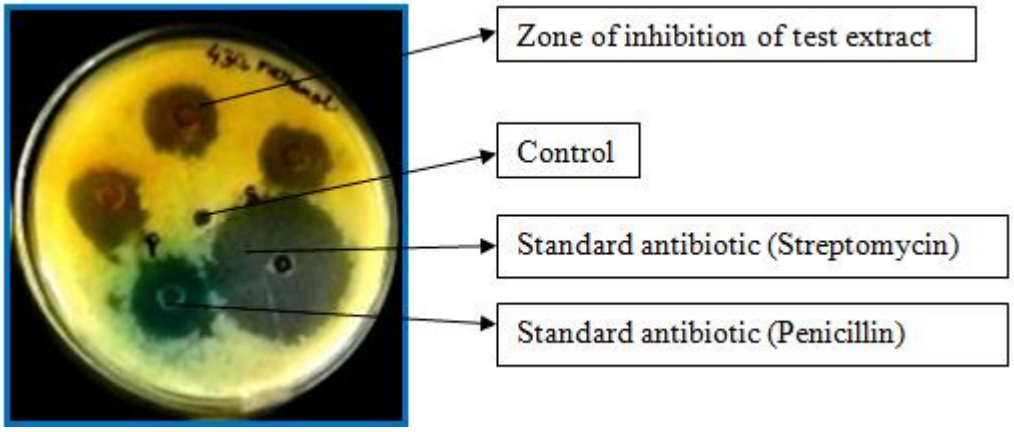

FIG. 2: ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACTS OF ROOT OF *C. CAESIA* AGAINST GRAM POSITIVE BACTERIA (*BACILLUS CERUS*)

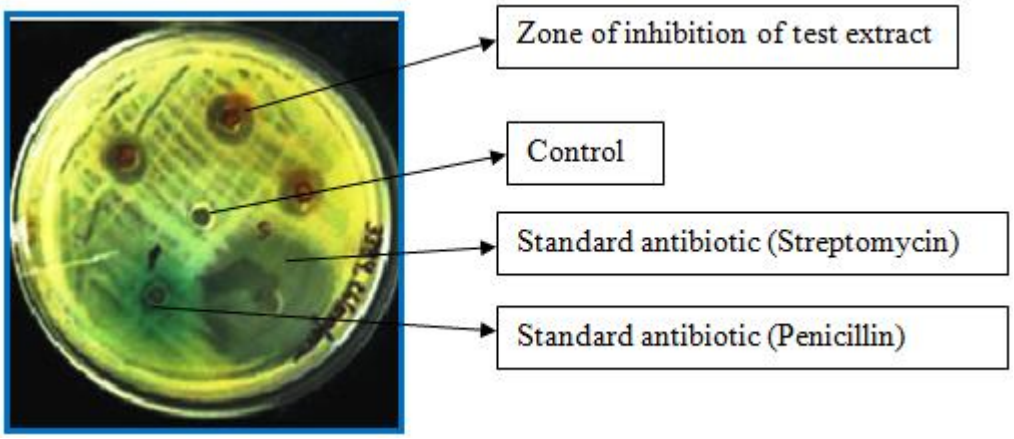

FIG. 3: ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACTS OF ROOT OF *C. CAESIA* AGAINST GRAM NEGATIVE BACTERIA (*KLEBSIELLA PNEUMONEAE*)

TABLE 4: ACTIVITY INDEX OF THE EXTRACT OF *CURCUMA CAESIA* IN DIFFERENT SOLVENTS WITH RESPECT TO STREPTOMYCIN

Bacterial Species ⇩	Chloroform			Acetone			Methanol		
	Root	Stem	Leaf	Root	Stem	Leaf	Root	Stem	Leaf
B. cerus	0.36	0.26	0.23	0.44	0.31	0.29	0.55	0.51	0.50
B. subtilis	0.37	-	-	0.41	-	-	0.53	-	-
S. aureus	0.31	0.25	0.24	0.42	0.29	0.29	0.46	0.37	0.36
S. epidermidis	0.45	0.28	0.27	0.50	0.42	0.31	0.54	0.47	0.46
E. coli	0.43	-	-	0.39	-	-	0.32	-	-
P. vulgaris	0.55	0.50	-	0.46	0.46	-	0.40	0.39	-
P. aeruginosa	-	-	-	-	-	-	-	-	-
K. pneumoneae	0.59	0.55	0.51	0.47	0.46	0.44	0.40	0.39	0.35

CONCLUSION: The present investigation was carried out to study the antibacterial potentiality in the root, stem and leaf extracts of *Curcuma caesia* in four different solvents against four gram positive and four gram negative bacteria. The results were promising and revealed that the methanol root extracts exhibited significant antibacterial activity against gram positive and chloroform root extracts against gram negative bacterial species under study except for *P. aeruginosa* followed by stem and leaf extracts. The above findings reveals that the plant based antimicrobials have enormous therapeutic potentials and can serve the purpose with lesser side effects that are often associated with synthetic antimicrobials. The present study would be a primary platform to explore local potential medicinal plants possessing antimicrobial efficacy and their further exploration proves to be the bedrock for future medicine.

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