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PHYTOCHEMICAL ASSESSMENT AND SYNERGISTIC BIOEFFICACY OF *CURCUMA CAESIA* (ROXB.) FROM BASTAR AGAINST MULTI-DRUG RESISTANT HUMAN PATHOGENS

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Keywords:

Phytochemicals, Bastar, Bioactive compound, Synergism, Multi-drug resistant, Human pathogens

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ABSTRACT: The current research is an attempt to investigate the antibacterial and synergistic bioefficacy of *Curcuma caesia* (Roxb.) versus human pathogenic bacteria *viz*., *Bacillus cereus*, *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus vulgaris* procured from IMTECH, Chandigarh, India. Agar well diffusion assay was performed, and one-way ANOVA examined the outcome. The qualitative phytochemical examination revealed a positive test for flavonoids, glycosides, phytosterols, resins, saponins, and tannins. However, the quantitative estimation of phytochemicals revealed that the root sample contains highest amount of flavonoid followed by total phenol, saponin and alkaloid. The bioactive extract was purified using column chromatography. The purified fraction and commercially available antibacterials *viz*., tetracycline, streptomycin, and penicillin was evaluated for their synergistic or antagonistic efficacy counter to multi-drug resistant human pathogenic bacteria. The outcome divulge that a purified fraction of *C. caesia* was found to act synergistically with tetracycline against all the bacterial cultures under investigation. The results with streptomycin showed maximum synergistic activity against *B. cereus*. However, penicillin and purified fraction exhibit utmost synergistic activity against *S. epidermidis* and *B. subtilis*. The results revealed that the methanolic root extract of *C. caesia* bears a potential bioactive phytocompound conferring enhanced synergism.

INTRODUCTION: The antibiotic resistance exhibited by multi-drug resistant human pathogenic microorganisms is a burning issue and is of serious global concern [1]. The clinical pathogenic bacterial strains possess the immense genetic potential to attain and transmit resistance against frequently used antibiotics [2, 3].

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The profuse use of antibiotics to cure infectious ailments induced the emergence of multi-drug resistant human pathogenic bacteria leading to a decline in health benefits over the past few decades [4, 5]. One of the best choices to combat this great resistance issue is the implication of combination therapy [6].

In the current scenario, combination therapy is a great boon to mankind, especially for patients suffering from severe infections due to MDR human pathogenic bacteria [7]. Synergism is a constructive interplay when two drugs amalgamate and employ an inhibitory outcome exceeding the sum of their discrete results [8].

Thus, in current scenario it is obligatory to investigate diverse types and number of medicinal plants conferring novel bioactive compounds which can be used in combination with standard antibiotics to cater a expanded spectrum of antibacterial activity and will also prove to be the milestone in laying the foundation of alternative combination therapy to target several infectious diseases for a better and healthy tomorrow. *Curcuma caesia* (Roxb.) belongs to Zingiberaceae [9, 10].

Commonly designated as "Black Turmeric" is an important and unexplored medicinal herb indigenous to Central India with bluish-colored rhizome [11]. The rhizome effuses a distinctive aroma on account of essential oil [12]. *Curcuma caesia* (Roxb.) possesses tremendous ethnomedicinal and economic value. The tribal inhabitants of Bastar have enormously employed it to ameliorate antimicrobial, anti-inflammatory, anticancer, and antiviral [13].

It also exhibits antioxidant, anti-tumour, antiasthmatic, anti-inflammatory, hepatoprotective, stomachic, and carminative properties [14]. Rhizomes are extensively used to cure bruises and sprains, smooth muscle relaxants, and cosmetics [15, 16]. Externally, it has been used for reducing inflammation and swelling [17].

Chhattisgarh is an herbal state possessing rich floral diversity, especially in Bastar [18]. Globally the Bastar district is well known for its Bastar is known for its idiosyncratic traditional knowledge, tribal culture, natural beauty, heritage, and medicinal plants. The traditional healers of Bastar have extensively used medicinal herbs to heal several infectious diseases since time immemorial.

Thus, due to the immense capability of medicinal herbs as a unique fountain of bioactive compounds, *Curcuma caesia* (Roxb.) was looked into to evaluate qualitative and quantitative photochemical composition along with synergistic antibacterial efficacy.

The sample collection and identification of *Curcuma caesia* (Roxb.) was performed at Shaheed Gundadhur College of Agriculture and Research Station (SGCARS), Kumhrawand, Jagdalpur, Chhattisgarh, India **Fig. 1.**

FIG. 1: MORPHOLOGY OF *CURCUMA CAESIA* (ROXB.)

MATERIALS AND METHODS: In central India, Bastar (19.1071°N, 81.9535°E) is a district of Chhattisgarh state **Fig. 2**. Bastar is situated at an elevation of 2000 ft plateau from sea level and has an area of 4029.98 km². Bastar district is divided into seven Tahsils *viz*., Jagdalpur, Bastar, Bakawand, Bastanar, Darbha, Lohandiguda and Tokapal. Bastar is well known worldwide for its tribal culture, and natural resources are enriched with natural beauty and a pleasant atmosphere favourable for the growth and cultivation of medicinal plants. Jagdalpur is the district headquarters of Bastar. Jagdalpur is a city surrounded towards the east by Navarangpur and Koraput, on the west by Narayanpur, the north by Kanker and Dhamtari and the south by Kondagaon.

FIG. 2: LOCATION OF BASTAR IN CHHATTISGARH, INDIA

Extraction Procedure: The extraction of phytochemicals was performed by Soxhlet apparatus **Fig. 3**. The preference of extraction mainly depends upon the target lead or bioactive compounds to be extracted [19]. The plant material, after shade drying was powdered using a laboratory

blender (Remi). The powdered plant sample was packed in a thimble and placed inside the extraction unit. The thimble was extracted sequentially one after the other in three different solvents *viz*., chloroform (non-polar), acetone (dipolar), and methanol (polar) [20, 21]. The powdered material and the solvent (1:10) were extracted according to their increasing polarity index in the Soxhlet apparatus (Tempo). The crude extract was dried in a hot air oven (Tempo), and the obtained residue was reconstituted in 50% Dimethyl sulfoxide for further synergistic antibacterial activity assessment.

FIG. 3: SOXHLET APPARATUS FOR EXTRACTION

Bacterial Cultures: Synergistic antibacterial bioefficacy was evaluated versus bacterial cultures procured from IMTECH, Chandigarh, India **Fig. 4**. The bacterial cultures along with their MTCC accession numbers were *Bacillus cereus* (MTCC 430), *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Staphylococcus epidermidis* (MTCC 435), *Escherichia coli* (MTCC 1687), *Klebsiella pneumoniae* (MTCC 3384), *Pseudomonas aeruginosa* (MTCC 741) and *Proteus vulgaris* (MTCC 744).

FIG. 4: HUMAN PATHOGENIC BACTERIAL CULTURES

Qualitative Phytochemical Analysis: The phytochemical evaluation for different solvent extracts of *Curcuma caesia* (Roxb.) was carried out qualitatively following the standard protocols [22, 23].

Quantitative Phytochemical Analysis:

Alkaloids: 100 ml of 20% acetic acid in ethanol was added to 2.5 g of powdered sample into a 250 ml beaker, mixed and kept to stand for 4 h. The above solution was then filtered, and the filtrate was concentrated using a water bath to about one-quarter of the actual volume. The filtrate obtained was then added to concentrated ammonia solution dropwise until the precipitation reaction was completed. The complete solution was allowed to settle. Finally, the precipitate was filtered with Whatman filter paper No. 42 and weighed to determine the amount of alkaloids present in sample [24].

Flavonoids: 50 ml of 80% aqueous methanol was added to 2.5 g of powdered sample into a 250 ml titration flask at room temperature and kept in an electric shaker for 4 h. The complete solution was filtered with Whatman filter paper No. 42. The above filtrate was then introduced into a crucible and kept over a water bath till dryness and weighed to determine the amount of flavonoids present in sample [25].

Saponins: 50 ml of 20% ethanol was added to 2.5 g of powdered sample. The above solution was kept in water bath at 55^{o}C with continuous stirring for 4 h. The residue and filtrate were re-extracted with 50 ml of 20% ethanol. The overall extracts so obtained were evaporated over a water bath at 90^{o}C to reduce the volume up to 20 ml. The concentrated extract was added with 10 ml diethyl ether into a 250 ml separating funnel and shaken strenuously. The ether layer was discarded, the aqueous layer was recovered, and 15 ml of n-butanol was added. The n-butanol extracts were washed twice with 5 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath till dryness and weighed to determine the amount of saponins present in sample [24].

Total Phenols: 50 ml n-hexane was added two times for 4 h each to 2.5 g of powdered sample into 250 ml conical flask. 25 ml diethyl ether was added twice, heated for 15 min each, cooled up to room

temperature, and filtered into a separating funnel. 25 ml of 10% sodium hydroxide solution was added two times and shaken well to separate the organic layer from the aqueous layer. It was washed thrice with 12.5 ml de-ionized water. The overall aqueous layer was acidified by adding 10% hydrochloric acid to a pH 4.0, and 25 ml dichloro methane was added two times to acidify the aqueous layer. Finally, the organic layer was collected, dried, and weighed to determine the amount of total phenols present in sample [24].

Synergistic Antibacterial Activity: The agar well diffusion method was used to evaluate synergistic antibacterial efficacy [26]. Petri plates containing 25 ml of Mueller Hinton Agar (Hi-media) were inoculated with standardized bacterial suspensions. The synergistic activity was assessed for purified methanol root fraction of *C. caesia* at 1 mg/ ml with standard antibiotics *viz*., tetracycline, streptomycin, and penicillin at 10 µg/ ml.

The petri plates containing agar were punched with wells (6mm) in diameter and 10 µl of purified methanol root fraction of *C. caesia* or antibiotics, and in case of synergistic or antagonistic effect with the purified methanol root fraction of *C. caesia* and the antibiotic was introduced into the well. Finally, all the petri plates were incubated at 37°C for 24 h in the incubator (Remi). The synergistic or antagonistic efficacy was assessed by measuring the zone of inhibition (mm). The mean of three replicates for each purified methanol root fraction of *C. caesia*, antibiotic and combination were computed. When combinations showed greater zone of inhibition (mm) than that of combined inhibition zone size by 0.5 mm, then the efficacy was regarded as synergistic activity [27].

Zone Size Interpretation: Bacterial cultures showing a clear zone of inhibition of (07-10 mm) was scored as non-inhibitory activity (Resistant), the zone of inhibition ranging (11-15 mm) was considered to be inhibitory activity (Sensitive) and ZOI greater than (16-20 mm) was considered as significantly inhibitory (antimicrobial activity) [28].

Statistical Analysis: The agar well diffusion assay and synergistic antibacterial efficacy assessment were performed in three replicates and the values are represented as Mean ± SE. The experimental findings were evaluated statistically using SPSS software version 16.0 by one-way ANOVA.

RESULTS: Qualitative phytochemical analysis of solvent extracts in different parts of *C. caesia* revealed that flavonoids were detected in all the parts in all extracts except chloroform stem and leaf extracts. A strongly positive reaction was recorded in methanol root extract. Phytosterols were found to be present in all the extracts, more so in methanol and acetone.

TABLE 1: QUALITATIVE PHYTOCHEMICAL ANALYSIS OF ROOT, STEM, AND LEAF EXTRACTS OF *C. CAESIA*

Tests	Methanol			Acetone			Chloroform		
	R	S	L	R	S	L	R	S	L
Alkaloids									
Mayer's	-	-	-	-	-	-	-	-	-
Wagner's	-	-	-	-	-	-	-	-	-
Hager's	-	-	-	-	-	-	-	-	-
Flavonoids									
Alkaline reagent	+++	++	+	++	+	+	+	-	-
Lead acetate	+++	++	+	++	+	+	+	-	-
Phytosterols									
Salkowski	++	++	++	++	++	+	+	+	+
Libermann-Burchard	++	++	++	++	++	+	+	+	+
Tannins									
Ferric chloride	++	+	-	+	+	-	-	-	-
Gelatin	++	+	-	+	+	-	-	-	-
Saponins									
Foam test	+	+	+	+	+	-	-	-	-
Quinones	-	-	-	-	-	-	-	-	-
Resins	+	+	+	+	+	+	+++	++	++
Glycosides	++	++	+	+	-	-	-	-	-

+++, Strongly positive; ++, moderately positive; +, positive; -, negative; R, Root; S, Stem; L, Leaf

Tannins were detected in methanol and acetone root and stem extracts. Saponins were found to be present in all extracts except chloroform of all the plant parts. Resins gave positive tests in all extracts, more so in chloroform. The glycosides were found to be present in methanol and to a less extent, in acetone extract but were completely absent in chloroform extracts. However, alkaloids and quinines gave negative tests in all the solvent extracts under investigation **Table 1**. The quantitative estimation of alkaloid, flavonoid, saponin, and total phenol in the root stem and leaf of *C. caesia* is presented in **Fig. 5**. The root contains the highest amount of flavonoid (1.147±0.017%) followed by total phenol (0.770±0.012%), saponin (0.540±0.020%) and alkaloid (0.330±0.015%). The flavonoids were recorded to be more in the root, followed by the stem and leaf. Total phenol was found to be (0.770±0.012%) in root, (0.517±0.031%) in stem and **(**0.397±0.029%) in leaf. Saponin was found to be (0.540±0.020%) in root, (0.385± 0.026%) in stem and (0.224±0.032%) in leaf. However, alkaloids were detected to be (0.330±0.015%) in the root, (0.219±0.017%) in the stem, and (0.180±0.024%) in the leaf.

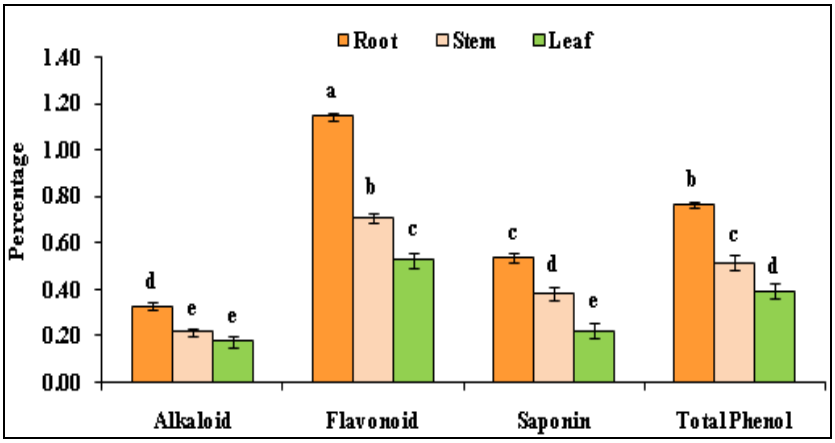

FIG. 5: ALKALOIDS, FLAVONOIDS, SAPONINS AND TOTAL PHENOLS IN ROOT, STEM AND LEAF OF *C. CAESIA* (ANOVA Summary: $F_{11, 24} = 133.47$, $p < 0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p < 0.001$) (Based on Duncan's multiple-range test).

The synergistic efficacy of purified methanol root fraction of *C. caesia* was evaluated with three antibacterials i:e. tetracycline, streptomycin & penicillin versus MDR human pathogenic bacteria cultures i:e. *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermidis*, *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *P. vulgaris*. The purified fraction of *C. caesia* in combination with tetracycline synergistically increased the antibacterial efficacy in contrast to when tested alone for Gram-positive bacteria.

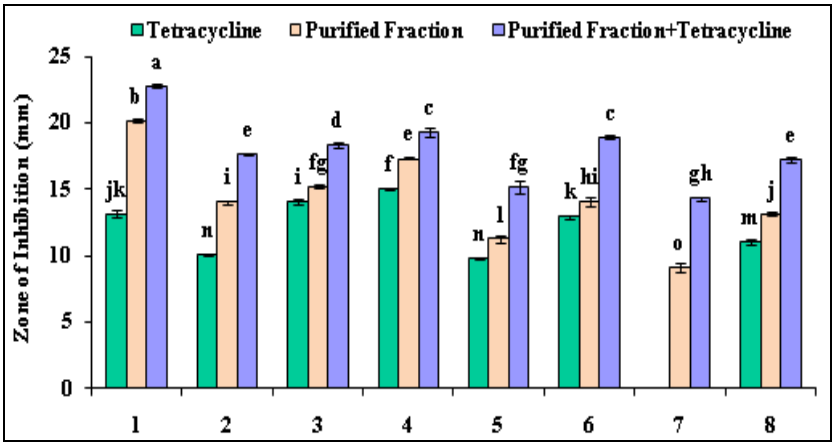

FIG. 6: SYNERGISTIC/ANTAGONISTIC POTENTIAL OF PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* WITH TETRACYCLINE AGAINST *B. CEREUS* (1), *B. SUBTILIS* (2), *S. AUREUS* (3), *S. EPIDERMIDIS* (4), *E. COLI* (5), *K. PNEUMONIAE* (6), *P. AERUGINOSA* (7), *P. VULGARIS* (8) (ANOVA Summary: $F_{23, 168} = 1051.00$, $p < 0.001$, Means having different alphabets, as superscripts are statistically significant from each other at $p < 0.001$) (Based on Duncan's multiple-range test).

The zone of inhibition (ZOI) was evaluated to be highest against *B. cereus* (22.80±0.11 mm) followed by *S. epidermidis* (19.33±0.37 mm), *S. aureus* (18.33±0.24 mm) and *B. subtilis* (17.66±0.06 mm). Although, for Gram-negative bacteria the maximum synergy with tetracycline was observed against *K. pneumoniae* (18.93±0.17 mm), followed by *P. vulgaris* (17.26±0.17 mm), *E. coli* (15.20±0.50 mm) and *P. aeruginosa* (14.33±0.13 mm). The results of the synergistic efficacy of the purified methanol root fraction of *C. caesia* with tetracycline are presented in **Fig. 6-7**.

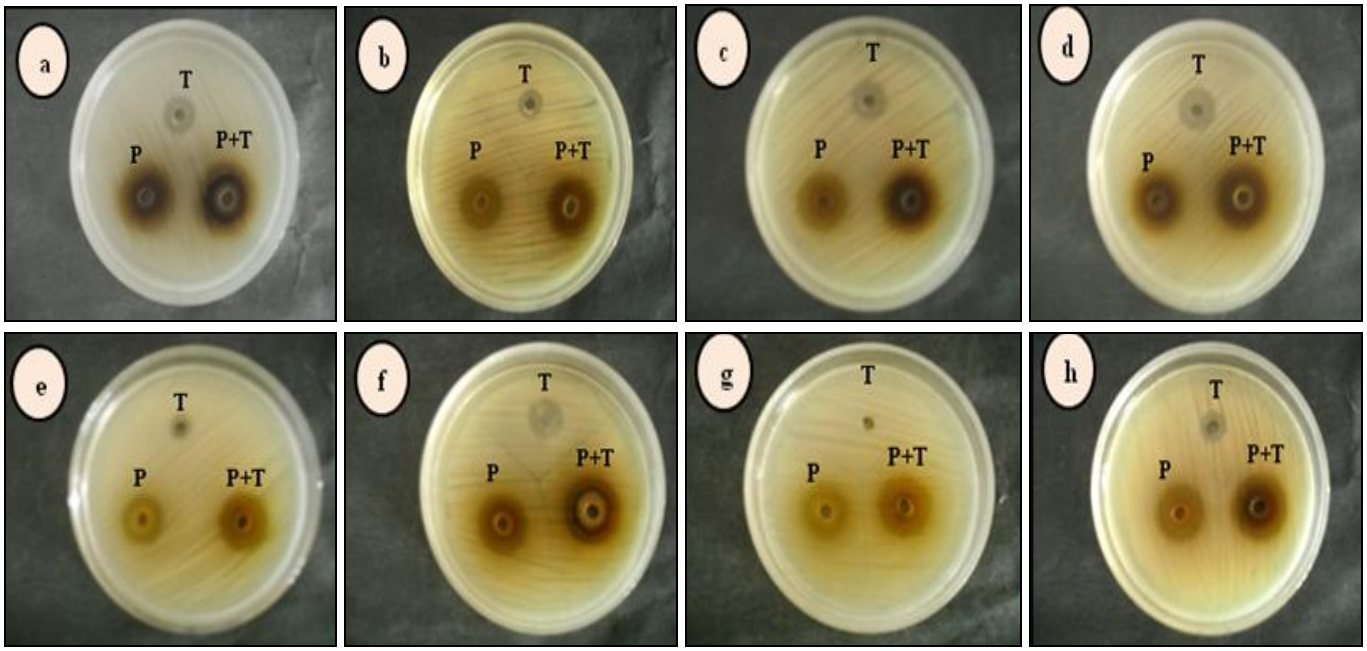

FIG. 7: SYNERGISTIC/ ANTAGONISTIC POTENTIAL OF PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* WITH TETRACYCLINE (A) *B. CEREUS* (MTCC 430), (B) *B. SUBTILIS* (MTCC 441), (C) *S. AUREUS* (MTCC 96), (D) *S. EPIDEMIDIS* (MTCC 435), (E) *E. COLI* (MTCC 1687), (F) *K. PNEUMONIAE* (MTCC 3384), (G) *P. AERUGINOSA* (MTCC 741), (H) *P. VULGARIS* (MTCC 744). (T- Tetracycline, P- Purified fraction and P+T- Purified fraction + Tetracycline).

The synergistic efficacy of purified methanol root fraction of *C. caesia* in combination with streptomycin revealed maximum synergistic activity against *B. cereus* with a zone of inhibition of 22.33±0.17 mm followed by *B. subtilis* (19.00±0.41 mm) whereas, antagonistic activity was recorded against *S. aureus* (13.26±0.06 mm) followed by *S. epidermidis* (14.00±0.11 mm).

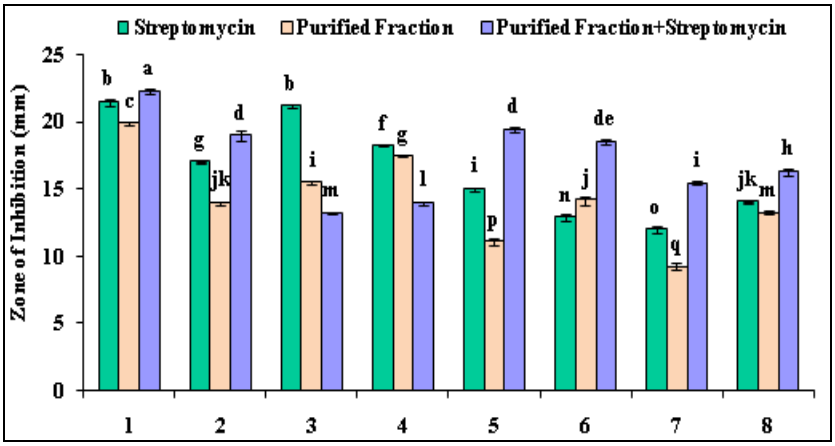

FIG. 8: SYNERGISTIC/ ANTAGONISTIC POTENTIAL OF PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* WITH STREPTOMYCIN AGAINST *B. CEREUS* (1), *B. SUBTILIS* (2), *S. AUREUS* (3), *S. EPIDERMIDIS* (4), *E. COLI* (5), *K. PNEUMONIAE* (6), *P. AERUGINOSA* (7), *P. VULGARIS* (8). (ANOVA Summary: $F_{23, 168} = 668.388$, $p < 0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p < 0.001$) (Based on Duncan's multiple-range test).

Although for Gram-negative bacteria the maximum synergy was recorded against *E. coli* (19.46±0.24 mm) followed by *K. pneumoniae* (18.53±0.17 mm), *P. vulgaris* (16.33±0.24 mm) and *P. aeruginosa* (15.46±0.13 mm). The results of the synergistic efficacy of the purified methanol root fraction of *C. caesia* with streptomycin are presented **Fig. 8-9**.

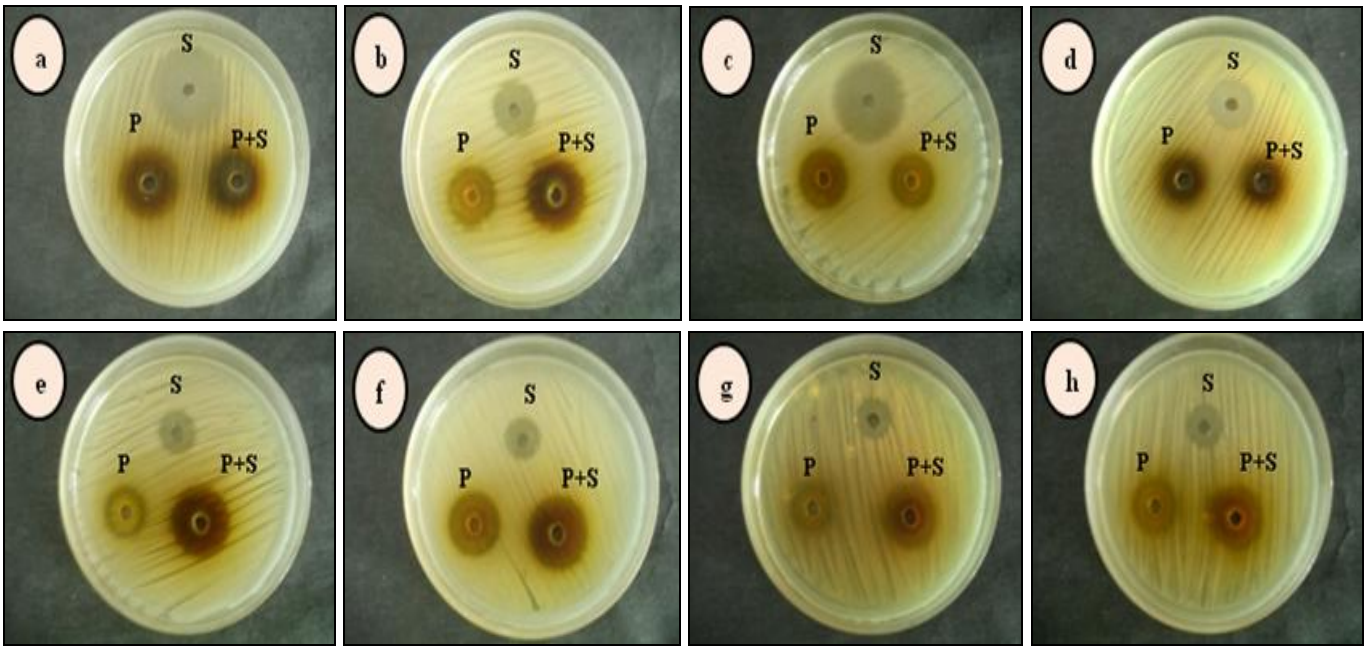

FIG. 9: SYNERGISTIC/ ANTAGONISTIC POTENTIAL OF PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* WITH STREPTOMYCIN (A) *B. CEREUS* (MTCC 430), (B) *B. SUBTILIS* (MTCC 441), (C) *S. AUREUS* (MTCC 96), (D) *S. EPIDEMIDIS* (MTCC 435), (E) *E. COLI* (MTCC 1687), (F) *K. PNEUMONIAE* (MTCC 3384), (G) *P. AERUGINOSA* (MTCC 741), (H) *P. VULGARIS* (MTCC 744). (S- Streptomycin, P- Purified fraction and P+S- Purified fraction + Streptomycin).

The synergistic efficacy of purified methanol root fraction of *C. caesia* along with penicillin revealed highest synergistic efficacy versus *S. epidermidis* with zone of inhibition 23.53±0.06 mm followed by *B. cereus* (22.46±0.24 mm), *S. aureus* (20.00±0.11 mm) and *B. subtilis* (18.26±0.17 mm). Although for Gram-negative bacteria all the combinations showed antagonistic activity. The ZOI for *P. aeruginosa* was 08.13±0.29 mm followed by *E. coli* (10.00±0.00 mm), *K. pneumoniae* (11.40±0.20 mm) and *P. vulgaris* (12.06±0.24 mm). The results of the synergistic efficacy of the purified fraction of *C. caesia* with penicillin are presented in **Fig. 10-11**.

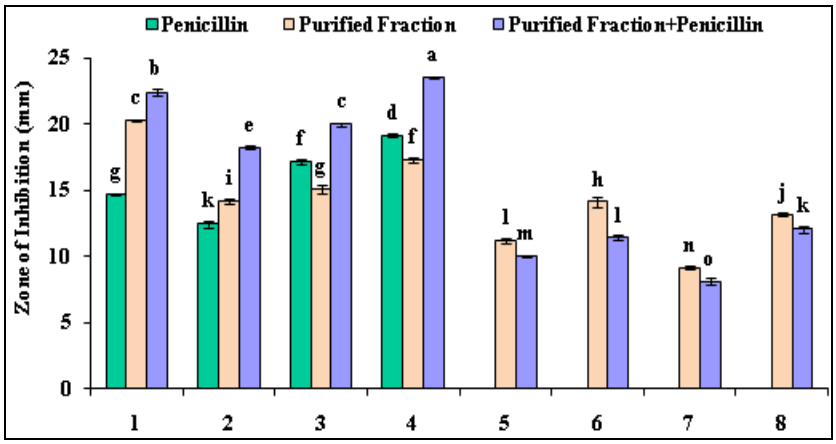

FIG. 10: SYNERGISTIC/ ANTAGONISTIC POTENTIAL OF PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* WITH PENICILLIN AGAINST *B. CEREUS* (1), *B. SUBTILIS* (2), *S. AUREUS* (3), *S. EPIDERMIDIS* (4), *E. COLI* (5), *K. PNEUMONIAE* (6), *P. AERUGINOSA* (7), *P. VULGARIS* (8). (ANOVA Summary: $F_{23, 168} = 3688.00$, $p < 0.001$, Means having different alphabets, as superscripts, are statistically significant from each other at $p < 0.001$) (Based on Duncan's multiple-range test).

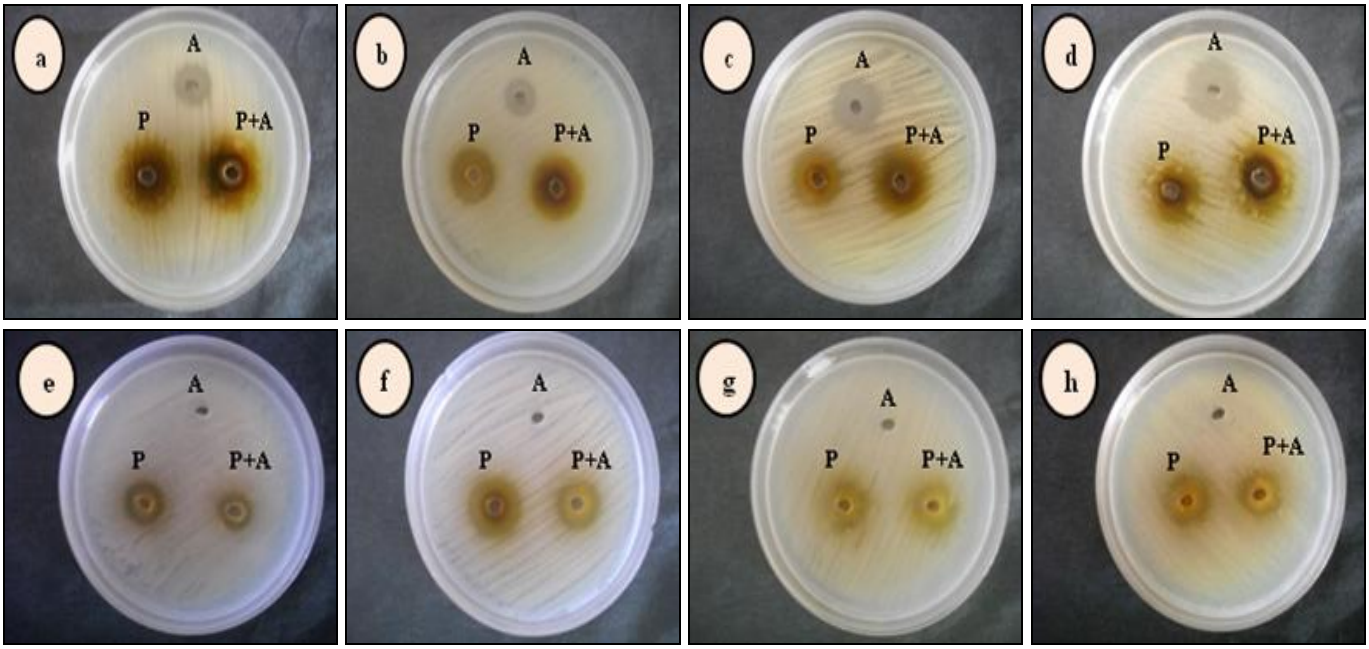

FIG. 11: SYNERGISTIC/ ANTAGONISTIC POTENTIAL OF PURIFIED METHANOL ROOT FRACTION OF *C. CAESIA* WITH PENICILLIN (A) *B. CEREUS* (MTCC 430), (B) *B. SUBTILIS* (MTCC 441), (C) *S. AUREUS* (MTCC 96), (D) *S. EPIDEMIDIS* (MTCC 435), (E) *E. COLI* (MTCC 1687), (F) *K. PNEUMONIAE* (MTCC 3384), (G) *P. AERUGINOSA* (MTCC 741), (H) *P. VULGARIS* (MTCC 744). (A- Penicillin, P- Purified fraction and P+A- Purified fraction + penicillin).

DISCUSSION: The qualitative phytochemical analysis of *C. caesia* methanol root extract revealed strong positive reaction for flavonoids, a moderate positive reaction for phytosterol, tannin and glycosides and a positive reaction for saponin and resins followed by its corresponding stem and leaf extracts whereas, acetone root extract exhibited moderately positive reaction for flavonoid and phytosterols but positive reaction for tannin, saponin, resins and glycosides subsequently by its stem and leaf extracts.

Chloroform root extract exhibited an extremely positive reaction for resins but less intense reaction for flavonoids and phytosterols. Similar findings depicting more bioactive in methanol root extract in comparison to its respective acetone extracts conferring strong antibacterial activity were also reported in some *Curcuma* sp. [29]. The quantitative study of alkaloid, flavonoid, saponin, and total phenol in root stem and leaf of *C. caesia* revealed that root contains the highest amount of flavonoid followed by total phenol, saponin, and alkaloid. The above findings agree with the research investigation by several researchers depicting the higher amount of flavonoids in roots [30, 31, 32, 33]. The plants produce flavonoids in response to microbial infections as hydroxylated phenolic substances, it possess the capability to complex with extracellular and soluble proteins and to complex with bacterial cell wall leading to cell death [34]. Thus, flavonoids were documented to possess higher antibacterial efficacy. The purified methanol root fraction of *C. caesia* was evaluated for its synergistic efficacy versus standard antibacterials i:e. tetracycline, streptomycin and penicillin.

The findings showed that tetracycline along with the purified methanol root fraction of *C. caesia* gave maximum antibacterial activity against *B. cereus* and comparatively less activity against *B. subtilis*. Although for Gram-negative bacteria, the maximum synergy was observed against *K. pneumoniae* and the minimum was recorded in the case of *P. aeruginosa*. The results suggested that the purified fraction was found to act synergistically with tetracycline against all the bacterial cultures under investigation. Tetracycline as the drug of choice for obtaining enhanced broad-spectrum synergistic antibacterial activity has been recorded [35, 36, 37]. The purified fraction of *C. caesia* with streptomycin showed maximum synergistic activity against *B. cereus*. However, it showed antagonistic activity against *S. aureus* and *S. epidermidis*. For Gram-negative bacteria, the maximum synergy was recorded against *E. coli* and

minimum against *P. aeruginosa*. The findings suggest that the combinations of purified methanol root fraction of *C. caesia* with streptomycin showed enhanced synergistic efficacy against all the bacterial cultures except *S. aureus* and *S. epidermidis*. The available literature documented the use of streptomycin with plant extracts showing a synergistic effect [38]. Likewise, penicillin and the purified methanol root fraction of *C. caesia* revealed maximum synergistic activity versus *S. epidermidis* and *B. subtilis*. Although for Gram-negative bacteria, all the combinations showed antagonistic activity. Several researchers have documented the synergistic or antagonistic efficacy of the combination of penicillin along with plant extracts [39, 40].

CONCLUSION: Combination therapy is a novel concept ameliorating multi-drug-resistant human pathogenic microorganisms. In the current scenario, the most commonly used antibiotics are comparatively less efficacious for clinical microbial infections because of multi-drug resistance among human pathogenic bacteria. Owing to this critical urgency, the present status has turned the attention of researchers and scientific community worldwide to look forward towards the alternative dimensions of healing infections. For that reason, the combination therapy with enhanced synergism is a beacon of light for patients suffering from diseases caused by MDR human pathogenic bacteria. Yet, *in-vitro* efficacy testing followed by several folds of clinical trials is required for the ultimate implication of this combination therapy. Therefore, in light of current prospects, the time is demanding to assess and evaluate many such potential medicinal herbs to isolate bioactive compounds conferring antimicrobial efficacy and further assess their synergistic potentiality along with its possible mode of action to enhance the antimicrobial spectrum with a lower dose and higher bioefficacy will definitely be rewarding for generations ahead and open new era of research in phytoscience for a better tomorrow.

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