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IN-VITRO ANTIMICROBIAL ACTIVITY OF *CURCUMA AMADA* ON THE CLINICAL ISOLATES OBTAINED FROM BURN'S WOUND

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Keywords:

Curcuma amada, Mango ginger, Cold extraction, Microwave Assisted Extraction and Soxhlet Extraction, Minimum Inhibitory Concentration, antipyretic

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ABSTRACT: The increasing resistance of microorganisms to conventional antibiotics has prompted scientists to explore alternative sources for antimicrobial compounds. This study focuses on *Curcuma amada*, a perennial herb, and its rhizome extracts for antibacterial activity against major bacterial strains identified in burn wounds: *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. Various extraction methods like Cold extraction, Microwave Assisted Extraction and Soxhlet Extraction were employed to obtain ethanolic and aqueous extracts and their effectiveness was evaluated through Minimal Inhibitory Concentration (MIC) testing. MIC, crucial in determining antimicrobial potency, revealed that the extract inhibited visible bacterial growth at a concentration of 100µg/ml for all tested organisms. This concentration was identified by a colour change from violet to pink, signifying the absence of observable microbial growth. Diverse medicinal properties of *Curcuma amada*, including anti-inflammatory, digestive, and febrifuge effects, present a promising avenue for developing natural antimicrobial agents. The findings suggest that these plant extracts possess potential as safe alternatives or complementary treatments in managing burn wounds. This research highlights the importance of exploring plant-derived compounds as a response to the urgent need for novel antimicrobial solutions against increasingly resistant microorganisms, particularly in the context of treating infectious diseases and wounds.

INTRODUCTION: Infectious diseases are the leading cause of death world-wide. Antibiotic resistance has become a global concern [1]. The flora of the burn wound also influence the risk of infection and the invasive potential of infections that do occur.

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The microbial population of the wound immediately after burning is sparse (bacteria in skin appendages commonly survive the burn) and predominantly gram-positive like *Staphylococcus aureus* which is the most common gram-positive early colonizer of the burn wound due to the use of penicillin.

These organisms can form myriad variably sized abscesses may also lead to dissemination of staphylococcal infections. The subsequent development and use of broad-spectrum antibiotics effective against *Staphylococcus* resulted in the

emergence of gram-negative organisms, particularly *Pseudomonas aeruginosa*, as the predominant organisms causing invasive burn wound infections in burn patients. Gram-negative organisms appear to have much greater invasive potential than gram-positive organisms due to toxins (both endotoxin and a variety of exotoxins), proteolytic enzymes, extracellular polysaccharides such as the slime produced by certain Pseudomonas organisms, and microbial motility imparted by a functioning flagellum. Along with these organisms, *Escherichia coli* and *Klebsiella pneumonia* can also be found the isolates obtained from the burn wounds which can cause fatal infections [2].

There are many infectious diseases which are known to be treated by using Herbal medicines or in combination with the modern medicines. Because of increasing resistance of organisms to the antibiotics, there is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore researchers are focusing more on plants and Phytochemistry [1]. Phytochemicals are the chemicals that present naturally in plants. Phytochemicals the bioactive non-nutrient plants compounds in fruit, vegetables, grains, and other plant foods have been linked to reductions in the risk of major chronic diseases. It is estimated that more than 5000 phytochemicals have been identified, but a large percentage still remain unknown and need to be identified before their health benefits are fully understood [34]. So, the researchers are now focusing on finding these compounds which have biological activity against some organisms that can be used to treat many bacterial infections [1].

Mango ginger (*Curcuma amada*) is a rhizomatous and perennial aromatic herb of the family Zingiberaceae and is cultivated throughout India, Sri Lanka, Bangladesh and in many South-East Asian countries for its rhizomes that are used as flavoring for pickles and other dishes and also valued for their medicinal properties [56]. *Curcuma amada* is a unique spice having morphological resemblance with ginger (*Zingiber officinale*) but imparts a raw mango (*Mangifera indica*) flavor. It is found wild, as well as in cultivation [7]. The rhizomes are bitter, sweet, sour aromatic (a mixture of tastes, starting from bitter initially, turning to a sweet and then sour aromatic sensation), and cooling; used as an appetizer, carminative, digestive, stomachic, demulcent, febrifuge, alexeteric, aphrodisiac, laxative, diuretic, expectorant, anti-inflammatory and antipyretic and used in the treatment of anorexia, dyspepsia, flatulence, colic, bruises, wounds, chronic ulcers, skin diseases, pruritus, fever, constipation, hiccough, cough, bronchitis, sprains, gout, halitosis, otalgia and inflammations [5]. The rhizomes are bitter, sweet, sour aromatic (a mixture of tastes, starting from bitter initially, turning to a sweet and then sour aromatic sensation), and cooling; used as an appetizer, carminative, digestive, stomachic, demulcent, febrifuge, alexeteric, aphrodisiac, laxative, diuretic, expectorant, anti-inflammatory and antipyretic and used in the treatment of anorexia, dyspepsia, flatulence, colic, bruises, wounds, chronic ulcers, skin diseases, pruritus, fever, constipation, hiccough, cough, bronchitis, sprains, gout, halitosis, otalgia and inflammations [5].

Antibacterial activity of a compound can be determined qualitatively or quantitatively. Well- and disc- diffusion methods have frequently been reported as qualitative indicators for testing the antimicrobial activity of natural products while Microdilution and agar dilution are quantitative methods that can be used to determine MIC values. The minimal inhibitory concentration (MIC), which is a key indicator of an antimicrobial agent's potency, is defined as the concentration (mg l-1) at which visible growth of bacteria is prevented under defined growth conditions. Agar dilution is a laborious, time consuming but the microdilution method is an accurate, inexpensive and easy to carry out. So this method is used to determine the MIC of the compounds which are naturally occurring [2].

In the present work, the antibacterial activity of different extracts of *Curcuma amad*a against the bacterial culture from burns isolates has been studied. Studies on different plants such as, *Mangifera indica* for their antimicrobial potential against few food pathogens such as *S. aureus* and *E. coli* have been reported but their role as food preservative is still not extensively evaluated. Also, the antimicrobial activity of *T. indica* has not been

evaluated against *L. monocytogenes* which may be potential against this noxious food pathogen. Studies on the extracts of *Curcuma amada* using different solvents like methanol, chloroform has been done previously. In this study, we have focusd on the ethanolic and aqueous extracts of *Curcuma amada* and their antibacterial activity.

MATERIALS AND METHODS:

Materials: In this work, Fresh and healthy mango ginger (*Curcuma amada* Roxb.) rhizomes **Fig. 1** were taken from local market of Dadar, Mumbai. The rhizomes were washed, sliced, dried in a hot air oven at 50°C for 36 h and then homogenized into fine powder. Two solvents (water and ethanol), and three extraction methods (Cold extraction, Soxhlet Extraction, Microwave Assisted Extraction) were used to evaluate their effect on the extraction yield [89].

FIG. 1: RHIZOMES OF *CURCUMA AMADA*

Extraction: For Cold Extraction, 20g of dry *Curcuma amada* powder was added to 200ml of 100% ethanol and distilled water (each solvent in two separate beakers) &; kept for 48 hours as shown in **Fig. 2**.

FIG. 2: COLD EXTRACTION USING ETHANOL AND DISTILLED WATER AS SOLVENT

Intermediate stirring was done for proper extraction. After 48 h it was filtered using Whatmann Filter paper No. 40. The solution obtained after filtration was poured in Petri plate and dried in Hot air oven at 40 °C for 72 h. Then the semi solid slurry obtained after drying was stored in eppendorf and stored at 4 °C [8, 10]. For Soxhlet Extraction, 20g of Powdered dried plant material was wrapped in a paper and added to the porous cellulose thimble. Following this, the solvent (200 ml of ethanol) is added to a round bottom flask, which is attached to a Soxhlet extractor and condenser on an isomantle. The crushed plant material is loaded into the thimble, which is placed inside the Soxhlet extractor. The solvent is heated using the isomantle at 65 °C and will begin to evaporate, moving through the apparatus **Fig. 3** to the condenser. This process is done for 35 cycles (approximately 20h). After the extraction, the solution is dried in hot air oven. Then the semi solid slurry is added to Eppendorf and stored at 4 °C [11, 6, 12].

FIG. 3: SOXHLET EXTRACTION USING ETHANOL AS SOLVENT

For Microwave Assisted Extraction, 20 g of powdered *Curcuma amada* was taken in a flask and 200 ml of distilled water was added to it. Both were mixed properly and then the flask was kept in the microwave oven at microwave power 160 W for 1 min. after 1 min the flask was removed and cooled for 1 min. This process was repeated further two times (i.e., heating for 1min and then cooling for 1 min). The solution was cooled and was filtered by using Whatman filter paper no. 40. The filtrate obtained was poured in a Petri dish was dried at 40°C for 72h. Dried semisolid slurry was transferred to an Eppendorf and kept at 4°C [13]. The extraction yield of selected plants has been calculated by the following equation [14].

$$\text{Yield (\%)} = (X1 \times 100) / X0$$

Where X1 refers to the weight of extract after evaporation of solvent and X0 refers to the dry weight of the plant powder before extraction. The extracts obtained from Cold extraction (ethanolic and distilled water extract), Soxhlet extraction (ethanol used solvent) and Microwave assisted extraction (solvent used distilled water) were checked for its sterility (i.e., to see whether the prepared extracts are sterile or not). 100mg of extract was taken in a fresh Eppendorf under sterile conditions and in the same Eppendorf 1 ml of sterile distilled water is pipetted. Both were mixed properly using a vortex and then a loopful of this solution is spread on Sterile Blood agar. This plate was then incubated at 37°C for 24 hours.

Preparation of Inoculum: *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumonia*, and *Staphylococcus aureus* were used as test organisms which were isolated from clinical burns isolate samples and obtained from the Burns Department, Bai Jerbai Wadia Hospital, Mumbai, India). The inoculum was prepared by using the protocol given by Sarker *et al.* Optical densities (OD) of the bacterial solutions were measured at 600 nm and sterile dilutions were made until the OD was in the range of 0.5- 1.0 [15].

Determination of Minimum Inhibitory Concentrations: The Minimum Inhibitory Concentration of the extract of *Curcuma amada* on four bacterial isolates was performed by Resazurin based Microtitre Dilution Assay. Under aseptic conditions, different concentrations of curcuma amada were employed (100 µg/ml -500 µg/ml). Control was maintained in duplicate. The colour change in the well was then observed visually. Any colour change occurred was recorded as the MIC of the test material [16, 17, 18, 15].

RESULTS AND DISCUSSION:

Extraction of Extracts and Yield Percentage of the Extracts: Four extracts of *Curcuma amada* were obtained by three extraction techniques viz. Cold Extraction, Soxhlet Extraction and Microwave Assisted Extraction using Ethanol and Distilled water as solvent. As shown in the Figure 4, Cold Extraction and Microwave Assisted Extraction using Distilled water as solvent gave a solid powder & Soxhlet Extraction and Cold Extraction using ethanol as solvent gave a semisolid slurry after drying the filtrate at 40°C for 72 h. All the extracts were stored at 4°C in refrigerator.

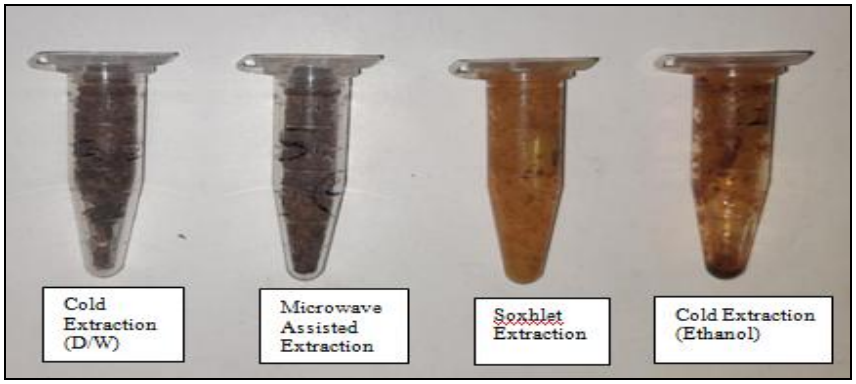

FIG. 4: EXTRACTS OF *CURCUMA AMADA* OBTAINED FROM DIFFERENT EXTRACTION TECHNIQUES

TABLE 1: EXTRACTION YIELD OF DIFFERENT EXTRACTS OF *CURCUMA AMADA*

Extraction method	Solvent used	Yield %
Cold extraction	Distilled water	2.03%
	Ethanol	3.25%
Microwave Assisted Extraction	Distilled water	5.04%
Soxhlet Extraction	Ethanol	8.81%

Table 1 summarizes Yield percentage of extracts of *Curcuma amada* obtained from three extraction method. Soxhlet extraction provided the highest yield of extract from the rhizomes of *Curcuma amada* which was found out to be 8.81%. This was more than the yield obtained from cold extraction (ethanol and distilled water is 2.03% and 3.25% respectively) & Microwave Assisted Extraction (5.04%). In Sterility checking, contamination was observed on the Sterile Blood agar plate in extracts derived from Cold Extraction (using Ethanol and Distilled water as solvents) and Microwave Assisted Extraction, as depicted in **Fig. 5B**. This signifies that the extract is not effective in killing the microorganisms rendering it unsuitable

for further study as practical application, such as incorporation into ointments or creams, requires sterile extracts. From these results, it can be said that either the protocol needs to be changed or there may have been some mistake while handling the extract. In case of extracts obtained from Soxhlet Extraction using ethanol as solvent, exhibited no contamination on the plate, as illustrated in **Fig. 5A** which indicates the suitability of the Soxhlet-extracted sample for further studies and its effectiveness in eliminating the microbes. Thus, it can be concluded that Soxhlet Extraction method is better than the other methods minimizing the likelihood of contamination during handling and yielding an effective extract for potential applications.

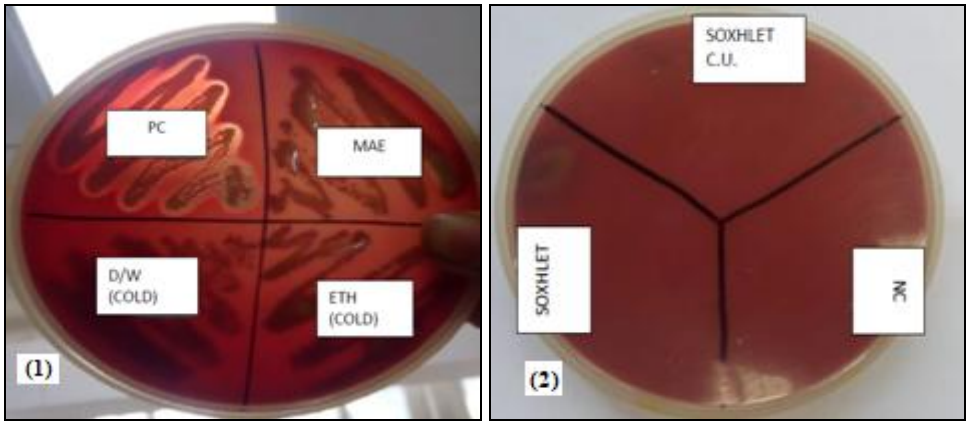

FIG. 5: STERILITY TESTING OF EXTRACTS OBTAINED FROM THREE EXTRACTION TECHNIQUES (COLD EXTRACTION, SOXHLET EXTRACTION & MICROWAVE ASSISTED EXTRACTION USING ETHANOL AND DISTILLED WATER AS SOLVENT

Determination of Minimum Inhibitory Concentration: The Minimum Inhibitory Concentration of the extract for the organisms *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Pseudomonas aeruginosa* was determined to be 100µg/ml, evident from the colour transition from violet to pink **Fig. 6**, denoting the concentration inhibiting visible microorganism growth. In a previous research [19], showed that the other extract like methanolic extract, chloroform extract, ethyl acetate extract had no antibacterial activity against *E. coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* but the Minimum Inhibitory Concentration performed by using ethanolic extract clearly showed antibacterial activity against them [17].

FIG. 6: DETERMINATION OF MINIMUM INHIBITORY CONCENTRATION OF SOXHLET EXTRACT OF CURCUMA AMADA ON FOUR MICROORGANISMS (*ESCHERICHIA COLI, STAPHYLOCOCCUS AUREUS, KLEBSIELLA PNEUMONIA* AND *PSEUDOMONAS AERUGINOSA*) ISOLATED FROM THE BURNS WOUND BY RESAZURIN METHOD. KEY: E = Escherichia coli, S = *Staphylococcus aureus,* K = *Klebsiella pneumoniae,* P = *Pseudomonas aeruginosa* C = Control

The results of this study provided an insight into the antibacterial properties of the extracts used traditionally for the prevention and treatment of many ailments like burns, ulcers and many more as

well as opportunity for selection of bioactive extracts for initial fractionation and further studies in antibacterial assays [18].

CONCLUSION: Researchers are focusing on the determining different phytochemicals and their uses because of the increasing demand for newer and newer medicines for the treatment of many infectious diseases. *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* were majorly found in the bacterial isolates isolated from the burns wound causing the infection in these patients. So, the aim of the study was to determine the antibacterial activity of Curcuma amada against the bacterial isolates. It has been proved in this research that the extract obtained has antibacterial activity against burn isolates which includes *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. This can be further used for preparing ointments and creams but before that there is a need for further testing (clinical studies).

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