

***In-vitro* Antibacterial and Antifungal Activity of *Curcuma Amada* Roxb. against Human Pathogens**

**Nand Kumar Kashyap¹, Jeetendra Deepak¹,
Arvind Kumar Bhardwaj¹, Milan Hait^{1,*} and Dilipkumar Pal²**

¹Department of Chemistry, Dr. C. V. Raman University, Kota, Bilaspur, C.G. 495113, India

²Department of Pharmaceutical Sciences, Guru Ghasidas Vishwavidyalaya, Koni, Bilaspur, C.G. 495009, India

(*Corresponding author's e-mail: haitmilan@gmail.com)

Received: 22 June 2022, Revised: 21 July 2022, Accepted: 30 July 2022, Published: 2 November 2022

Abstract

This investigation was conducted to evaluate the antimicrobial effectiveness of the agar disc diffusion method and to determine the inhibition zone of *Curcuma amada* (CA) rhizome extracts (petroleum ether, dichloromethane, and chloroform) against 12 bacterial species and 4 fungal species. The inhibition zones of the extracts were compared to those of Ciprofloxacin and Griseofulvin for antibacterial and antifungal activities, respectively. The findings revealed that the organisms under examination showed significant inhibition of bacterial as well as fungal development during the experiment and that the typical form of *C. amada* rhizome can be used to treat bacterial and fungal illnesses. So, this plant could be used to find natural compounds that are bioactive and could be used to make new medicines.

Keywords: *Curcuma amada*, Rhizome, *In-vitro*, Antibacterial, Antifungal

Introduction

Infections and illnesses produce social and economic problems all around the world. In the pathophysiology of infection illnesses, microbial origins play a key part, and as the medicine of choice for the treatment of infections caused by microorganisms, antibiotics are widely used. Currently, a wide variety of antibiotics are used to treat bacterial illnesses, including penicillin, amoxicillin, cephalosporin, sulphonamide, Cefepim, tetracycline, chloramphenicol, gentamicin, quinolones, and ciprofloxacin, among other medications [1,2]. Since they were first discovered, the public has been safer because antibiotics have been used to fight bacterial illnesses more powerfully than any other class of drugs. However, over the last several decades, these health benefits have become quite efficient against specific diseases, mostly because several of them have harmful side effects, but also because of the development of drug-resistant bacteria. This is due to the development of antibiotic-resistant bacteria. It is critical to look into innovative therapies that are less susceptible to reactions in order to advance the field. In the prevention and treatment of a broad variety of human illnesses, herbal medicines and other natural sources are essential. Conventional medicine is a basic health system in many impoverished nations, and it is one of the most effective. In the past, herbs were used a lot in herbal medicine, and their healing properties have been well-known. The experimental medicines that have been produced recently are based on natural components, and they have been shown to be quite effective, particularly in the fields of acute disease and cancer prevention and treatment. Natural bioactive compounds that come from plants could be a good source of antimicrobial drugs. Some of these compounds could have new ways of working that aren't found in traditional antimicrobial drugs.

The benefits of herbal extracts upon microorganisms have already been analyzed by a large majority of knowledge in various distinct places around the globe, and the results have been recorded. The medicinal plants are effective for repairing as well as treating human ailments due to the existence of phytochemical components. Plant extracts are applied as local medications in many regions of the globe to fight different pathogenic illnesses. Due to the high resistance of many microbes against the presently commercially available antibiotics, analysis of the bioactive molecules in medicinal plants has become necessary. Throughout ancient times, antimicrobial drugs, which originated from plants, have been used to protect the safety of food since the dawn of civilization. Natural products are being investigated to see whether they

might be used to boost health and development due to growing concerns about antimicrobial resistance. Plants can generate phytochemicals in virtually infinite quantities to protect themselves from microbes, insects, and herbivores. Many molecules contribute to plant flavour, and certain herbs and spices used to season meals also contain medicinal components.

Mango ginger (*Curcuma amada*) is a remarkable perennial herbaceous plant whose rhizomes have a physical similarity to ginger but which has a distinct mango flavour. Mango ginger is a popular item in India. Rhizomes are golden brown in colour, bear certain layers, and are used in food. *C. amada* belongs to the Zingiberaceae family. *C. amada* has a huge amount of potential as a medicinal herb, colourant, and flavouring. In addition to being used in the making of pickles, *C. amada* is also used as a source of raw mango flavour in meals and as a medicinal herb. The rhizome is important in Ayurveda, India's most important system of medicine, because it is an appetiser, alexteric, antipyretic, aphrodisiac, laxative and many more [3-5].

The microbes are posing a challenge to scientists by increasing resistance to the medications that are now accessible. There are several antibacterial and antifungal agents available today, all of which have been produced by scientists. Plants have been found to create a number of chemicals to defend themselves against a wide range of infections, and as a result, they are regarded as being a possible source for a range of antimicrobial agents of various types. As a result, the present investigation was performed in order to determine the antibacterial activity of *C. amada* rhizome extracts. *C. amada* has long been valued for its therapeutic potential. Antifungal and antibacterial activity of *C. amada* has been observed in some investigations [2].

In order to emphasize the importance of *C. amada*, which is used as a functional food supplement and herbal remedy component, it is possible to explore its effectiveness against various pathogens, and it could be a better antimicrobial agent, extracted from *C. amada* rhizomes collected from Pamgarh (Chhattisgarh) and designed to give a deeper direction for achieving the more beneficial advantages of the *C. amada* rhizome in the future.

Materials and methods

Sample collection

Curcuma amada (Mango-ginger) was collected from the local home garden at Pamgarh, District Janjgir-Champa, Chhattisgarh, India. The rhizomes (**Figure 1**) were cleaned, cut into small pieces, and dried in the shade, then crushed into a coarse powder using a mortar and pestle.

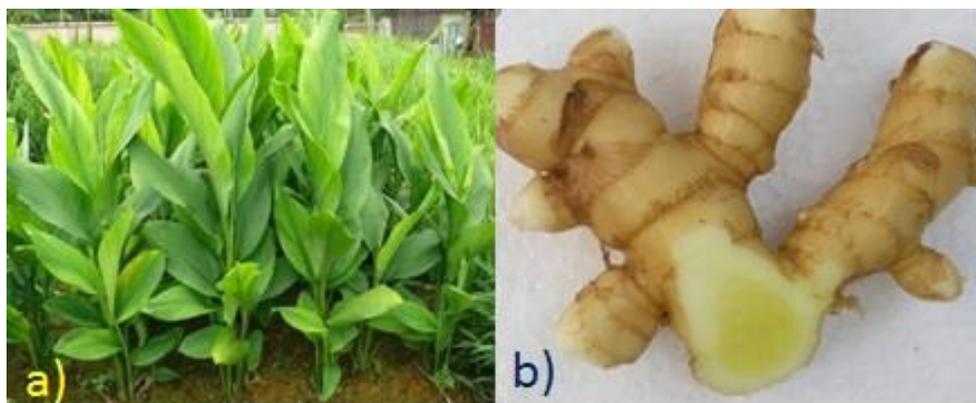


Figure 1 Plant of *Curcuma amada*: a) leaf and b) rhizome.

Preparation of plant extracts

Curcuma amada (CA) rhizome parts were gently cleaned and dried in a shaded place for a few days. The shade-dried rhizomes of *C. amada* were ground into a roughly powdered form using a mortar and pestle and stored in a sealed glass vial for future uses. 200 g of shade-dried, roughly powdered *C. amada* rhizome were extracted successively with petroleum ether (Pet. ether), dichloromethane (DCM), and chloroform in a Soxhlet extractor at 50 - 60 °C. A rotary evaporator was used to concentrate the extract at 40 °C. Extracts were kept at 4 °C for future analysis. The solvent ratio was chosen based on its polarity nature and previously published procedures [6,7].

Collection of pathogen

The microorganisms for the antimicrobial tests were collected from Guru Ghasidas Central University in Bilaspur, Chhattisgarh, India. A total of 12 bacterial species were collected, including *Escherichia coli* (NCTC 7360, 5933, and K88), *Salmonella typhi*, *Salmonella enterica*, *Shigella dysentery*, *Shigella sonnei*, *Staphylococcus aureus*, *Bacillus subtilis*, *Bacillus pumilus*, *Vibrio cholerae* (NCTC 4693), *Vibrio cholerae* (NCTC 10732), and 4 fungal species, including *Candida albicans*, *Aspergillus niger*, *Penicillium notatum*, and *Penicillium funiculosum* were employed in this study. Agar medium was used to grow bacteria, while Sabourauds dextrose agar was used to grow fungus.

Culture media

The nutrient agar solution (NAS) and Sabouraud's dextrose agar solution (SDAS) were purchased from Hi Media Laboratories Ltd., Mumbai. To make the malt, glucose, yeast, peptone, and agar mixture solution, we boiled 1 liter of distilled water, 5 g of malt extract, 10 g of glucose, 5 g of yeast extract, 5 g of peptone, and 20 g of agar. The solution was maintained at a pH of 6.4 - 6.8 and inoculated for 30 min.

Phytochemical screening

Phytochemical screening was done using our previously described methods [8,9]. The CA rhizome was analyzed through a qualitative approach using a few distinct, well-established analytical processes in order to investigate the various natural bioactive components that were found within it. The Salkowski test for terpenoids, the ferric chloride test for tannins, the Liebermann-Burchard test for sterol, the Shinoda test for flavonoids, the Molisch test for carbohydrates, the Wagner test for alkaloids, the ninhydrin test for amino acids, the Killer Killiani test for glycosides, the ferric chloride test for phenols, and the precipitate test for phlobatannin. With the exception of oxalates, quinones, and phlobatannin, most of the results were confirmed.

Screening for antimicrobial activity

The agar-disc diffusion technique was applied to investigate antimicrobial activity. Various concentrations of the extracts were made with petroleum ether, dichloromethane (DCM), and chloroform solvent, and all the extracts were rebuilt with dimethylsulphoxide (DMSO). One mL of 1-day-freshly-cultured bacteria and 1 mL of 2-day-freshly-cultured bacteria were carefully mixed with 10 mL of sterile melting agar that had been cooled to about 40 °C. Then, the agar was spread on the agar plates. A sterile borer was used to make four 6 mm diameter wells once the material had hardened. 75 mL of sample extracts or solvent blanks were poured into the wells. For 1-day, antibacterial test plates were kept at 35 °C. Antifungal test plates were kept at room temperature for 2 days. The antibiotic Ciprofloxacin was employed as a positive antibacterial reference drug, whereas Griesofulvin was applied as a positive antifungal reference drug, respectively. In this study, the diameter of each disc's bacterial inhibition zone was used to measure its antimicrobial power. Each test was completed in triplicate, and the average diameter of the inhibitory zone was measured [10-12].

Determination of MIC

In order to determine the minimal inhibitory concentration for the tested microorganisms, several concentrations of extracts were used, with DMSO serving as a negative control. The concentrations of extracts ranged from 5 to 800 µg/mL. Approximately 65 µL of each concentration was placed in each well of the agar plate. Then, the wells were scrubbed with freshly grown and tested microorganisms. Bacteria were kept at 30 °C for 1 day, and fungal pathogens were kept at 40 °C for 2 days. MICs were calculated by comparing each treatment with each other.

Determination of antimicrobial activity

The agar disc diffusion technique was used to test *in-vitro* antibacterial activities. After overnight growth, the bacterial cultures were centrifuged at 5,000 rpm for 5 min and deposited in saline. It was then placed in a sterilized petri dish plate and combined with melted Mueller Hinton agar media before being allowed to set. A 6 mm well was formed in pre-seeded Muller Hinton agar plates. A 50 µL sample was placed in each well and diffused for 2 h. The diameter of the inhibitory zones was measured after 1 day of incubation at 37 °C. The average of the 3 wells was used to determine each extract's growth inhibition zone. For each bacterial strain, a well was infected with pure solvent (DMSO). The control zone was subtracted from the test zone, and the results were graphed. The test was done in triplicate. DMSO was employed as a negative control. Ciprofloxacin and Griesofulvin were used as positive controls [13-18].

Table 1 Phytochemical screening of *C. amada* rhizome [9].

S. N.	Phytochemicals	Solvent extracts		
		Pet. Ether	DCM	Chloroform
1	Alkaloids	+	+	+
2	Cardiac Glycosides	-	+	+
3	Carbohydrates	+	+	+
4	Flavonoids	-	+	+
5	Phenols	-	+	-
6	Phlobatannins	-	-	-
7	Proteins	-	+	-
8	Saponins	+	-	+
9	Sterols	+	-	+
10	Tannins	+	+	+
11	Terpenoids	+	+	+
12	Quinones	-	-	-
13	Oxalates	-	-	-

+ = Present; - = absent; Pet. ether = petroleum ether; DCM = Dichloromethane

Results and discussion

According to the findings of the qualitative phytochemical screening, the extract of *C. amada* contains a wide variety of phytochemicals, including carbohydrates, flavonoids, alkaloids, cardiac glycosides, phenols, saponins, sterols, tannins, and terpenoids, etc. For this, it is reasonable to assume that the CA rhizome includes a variety of distinct phytochemicals, which are outlined in **Table 1**. The antimicrobial efficacy of various solvent extracts of *Curcuma amada* rhizome was tested. The antibacterial activity was tested in petroleum ether, DCM, and chloroform extracts against various strains of *E. coli*, *Salmonella typhi*, *Shigella dysentery*, *Staphylococcus*, *Bacillus*, *Vibrio cholera*. The agar disc-diffusion method was used for the investigation of antibacterial properties, and the findings are reported in **Tables 2** and **3**. We tested the antimicrobial activity of Petroleum ether, DCM, and Chloroform crude extract of *Curcuma amada* rhizomes in concentrations ranging from 5 to 800 g/mL against a 12 variety of pathogenic bacterial strains, including *E. coli* (NCTC 5933, K88, and NCTC 7360), *Salmonella typhi* Ty2, *Salmonella enterica* TD 01, *Shigella dysentery*, *Shigella sonnei* 1, *Staphylococcus aureus* ML 267, *Bacillus pumilus* 82, *Bacillus subtilis* ATCC 6633, *Vibrio cholerae* NCTC 4693 and *Vibrio cholerae* NCTC 10732, and 4 fungal strains, including *Candida albicans* ATCC 10231, *Aspergillus niger* ATCC 6275, *Penicillium notatum* ATCC 11625, and *Penicillium funiculosum* NCTC-287. *Staphylococcus aureus* ML-267 and *Escherichia coli* NCTC were used to Gram positive and Gram negative, respectively.

Table 2 Bacterial zone of inhibition of *C. amada* extract and Ciprofloxacin at 200 µg/mL.

S. N.	Bacteria	Inhibition zone diameter (mm)			
		Pet. ether	DCM	Chloroform	Ciprofloxacin
1	<i>E. coli</i> NCTC 5933	14	13	14	16
2	<i>E. coli</i> K88	14	13.5	14.5	17
3	<i>E. coli</i> NCTC 7360	14.5	14	14.5	17
4	<i>Salmonella typhi</i> Ty2	14	14	13	16
5	<i>Salmonella enterica</i> TD 01	14.5	15	13.5	19
6	<i>Shigella dysentery</i> 8	14	14.5	13.5	20
7	<i>Shigella sonnei</i> 1	14	14	13	19.5
8	<i>Staphylococcus aureus</i> ML 267	15	17	16	18

S. N.	Bacteria	Inhibition zone diameter (mm)			
		Pet. ether	DCM	Chloroform	Ciprofloxacin
9	<i>Bacillus pumilus</i> 82	7.5	8	8	19
10	<i>Bacillus subtilis</i> ATCC 6633	7.5	8	8	18
11	<i>Vibrio cholerae</i> NCTC 4693	12	13	15	17.5
12	<i>Vibrio cholerae</i> NCTC 10732	13	13	14.5	19

DCM = Dichloromethane; *E. coli* = *Escherichia coli*

These strains have been chosen because they can be used for more formulation research. It was Ciprofloxacin and Grieseofulvin that were used as the standard drugs, each with 10 mg/mL of each. The zones of bacterial growth inhibition were measured to determine the antibacterial and antifungal properties of each extract.

Table 3 Mode of antibacterial action at 200 µg/mL.

S. N.	Bacteria	Mode of action		
		Pet. ether	DCM	Chloroform
1	<i>E. coli</i> NCTC 5933	BC	BC	BC
2	<i>E. coli</i> K88	BC	BC	BC
3	<i>E. coli</i> NCTC 7360	BC	BC	BC
4	<i>Salmonella typhi</i> Ty2	BC	BC	BC
5	<i>Salmonella enterica</i> TD 01	BC	BC	BC
6	<i>Shigella dysentery</i> 8	BC	BC	BC
7	<i>Shigella sonnei</i> 1	BC	BC	BC
8	<i>Staphylococcus aureus</i> ML 267	BC	BC	BC
9	<i>Bacillus pumilus</i> 82	BS	BS	BS
10	<i>Bacillus subtilis</i> ATCC 6633	BS	BS	BS
11	<i>Vibrio cholerae</i> NCTC 4693	BC	BC	BC
12	<i>Vibrio cholerae</i> NCTC 10732	BC	BC	BC

BS = Bacterostatic; BC = Bactericidal; *E. coli* = *Escherichia coli*

Tables 2 to 5 and **Figures 2 to 3** exhibit the findings of antibacterial and antifungal activity, respectively. Each time the concentration of extracts was increased, the antibacterial and antifungal properties of all of the extracts rose at the same rate. When contrasted to reference drugs, the findings confirmed that *E. coli*, *Solmonella typhy*, *Shigella dysentery*, and *S. aureus* were more receptive to the extracts when tested for bacterial activity. *Vibrio cholera* and other pathogens were less receptive. In fungal activity, *A. niger*, *P. notatum*, and *P. funiculosum* show better results than *C. albicans*. It varied between 10 and 20 mm in diameter for all of the microorganisms that were responsive. In the case of fungal strains, the diameter varied from 10 to 20 mm (**Figure 3**). According to the findings, *Curcuma amada* rhizome extracts have been found to be very effective against all of the microorganisms that were tested.

Table 4 Fungal zone of inhibition of *C. amada* extracts at 1,500 µg/mL of Fungi.

S. N.	Name of Fungi	Inhibition zone diameter (mm) sample drug in Sabarauds Dextrose agar media			
		Pet. ether	DCM	Chloroform	Griseofulvin
1	<i>Candida albicans</i> ATCC 10231	11	12	12	15
2	<i>Aspergillus niger</i> ATCC 6275	12.5	13	13.5	14.5
3	<i>Penicillium notatum</i> ATCC 11625	13.5	11.5	10.5	13
4	<i>Penicillium funiculosum</i> NCTC 287	13	12	10	13

DCM = Dichloromethane

Table 5 Mode of antifungal action of *C. amada* extracts at 1,500 µg/mL.

S. N.	Name of Fungi	Pet. ether	DCM	Chloroform	Griseofulvin
1	<i>Candida albicans</i> ATCC 10231	FS	FC	FC	FC
2	<i>Aspergillus niger</i> ATCC 6275	FC	FC	FC	FC
3	<i>Penicillium notatum</i> ATCC 11625	FC	FC	FC	FC
4	<i>Penicillium funiculosum</i> NCTC 287	FS	FC	FC	FC

FS = Fungistatic; FC = Fungicidal

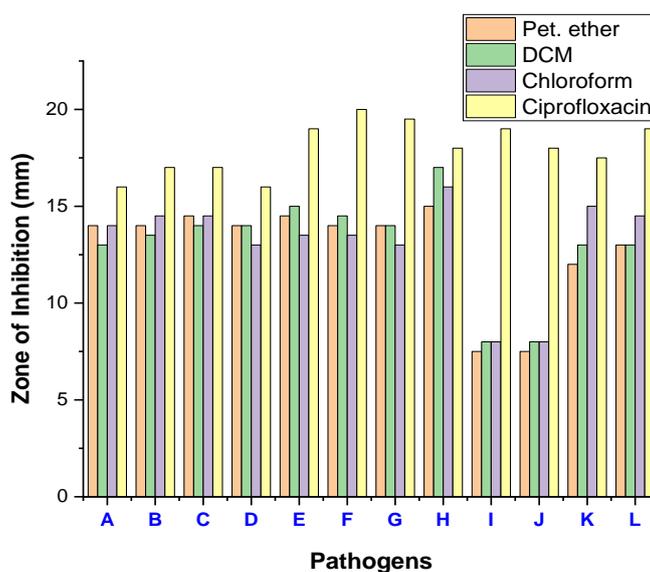


Figure 2 Bacterial zone of inhibition of *C. amada* rhizome extracts and Ciprofloxacin at 200 µg/mL. **A** = *E. coli* NCTC 5933, **B** = *E. coli* K88, **C** = *E. coli* NCTC 7360, **D** = *Salmonella typhi* Ty2, **E** = *Salmonella enterica* TD 01, **F** = *Shigella dysentery* 8, **G** = *Shigella sonnei* 1, **H** = *Staphylococcus aureus* ML 267, **I** = *Bacillus pumilus* 82, **J** = *Bacillus subtilis* ATCC 6633, **K** = *Vibrio cholerae* NCTC 4693, **L** = *Vibrio cholerae* NCTC 10732.

The antibacterial properties of herbal medicines have been rapidly discovered in a variety of locations across the globe, including India. According to the WHO, plant parts and their bioactive natural compounds are employed as herbal remedies in therapeutic approaches by 70 % of the global population, and this figure is conservative. In this research work, the extracts prepared from *Curcuma amada* displayed significant efficacy against the majority of the tested microorganisms. The findings were

similar to the antibiotics used as a control group. The findings of this research provide considerable proof that *C. amada* rhizome is a potent antibacterial agent. Extracts of *C. amada* rhizome were shown to be efficacious against all organisms tested, including antibacterial strains, fungal strains, which were receptive to all extracts of *C. amada* rhizome. The findings show that *C. amada* extracts in petroleum ether, DCM, and chloroform have considerable antibacterial and antifungal power.

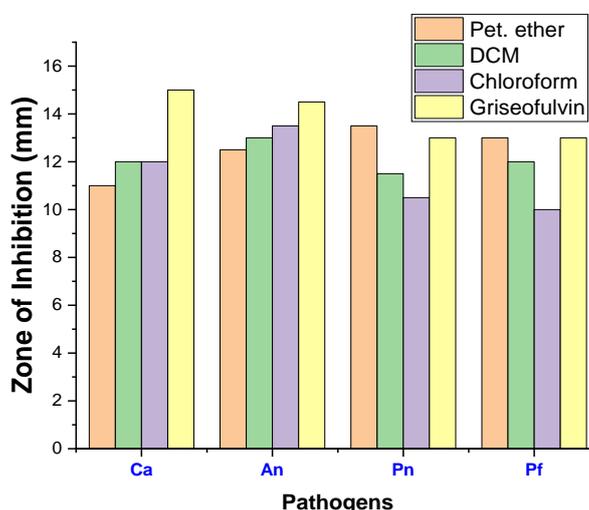


Figure 3 Fungal zone of inhibition of *C. amada* extracts at 1,500 µg/mL. **Ca** = *Candida albicans* ATCC 10231, **An** = *Aspergillus niger* ATCC 6275, **Pn** = *Penicillium notatum* ATCC 11625, **Pf** = *Penicillium funiculosum* NCTC 287.

Conclusions

Curcuma amada contains a high concentration of secondary metabolites having antifungal and antibacterial properties. The satisfactory findings were obtained with all the extracts, which will be useful in the future when conducting MIC as well as other formulation studies. In contrast to traditional medicines, *C. amada* extracts have been found to be effective against most of the microorganisms (bacteria and fungi) that are used to treat illnesses. It was found that the rhizome part of *C. amada* was applied in conventional medicine to cure numerous infectious diseases caused by bacteria, which supported the claims of the researchers. More research is required in order to more accurately assess the possible effectiveness of plant extracts as antibacterial and antifungal agents. It is hoped that the current findings will serve as the foundation for the identification of plant species for future inquiry into discovering novel natural bioactive chemicals. Further research will have to be done into the plant's antibacterial active components, including the identification and structural characterization of these compounds, as well as how they work.

Acknowledgements

The authors of this paper are thankful to the HOD, Department of Chemistry, Dr. C. V. Raman University, Kota, Bilaspur, Chhattisgarh, India, for valuable support and providing all the research facilities.

References

- [1] AK Al-Ghamdi, TA Elkholy, S Abuhelal, H Al-Abadi, D Qahwaji, N Khalefah, H Sobhy and M Abu-Hilal. Phytochemical investigation and *In-vitro* anthelmintic activity of the leaves of *Gynura lycopersifolia* Linn. *Pharmacogn. J.* 2019; **11**, 191-4.
- [2] LY. Zhang, JW Wei, ZW Yang, F Chen, QQ Xian, P Su, WY Pan, K Zhang, X Zheng and ZY Du. Distribution and diversity of twelve *Curcuma* species in China. *Nat. Prod. Res.* 2017; **32**, 327-30.

- [3] Y Chen, MK Shukurova, Y Asikin, M Kusano and KN Watanabe. Characterization of volatile organic compounds in mango ginger (*Curcuma amada* Roxb.) from Myanmar. *Metabolites* 2021, **11**, 1-10.
- [4] S Jena, A Ray, A Sahoo, PC Panda and S Nayak. Deeper insight into the volatile profile of essential oil of two *Curcuma* species and their antioxidant and antimicrobial activities. *Ind. Crops Prod.* 2020; **155**, 1-11.
- [5] R Mahadevi and R Kavitha. Phytochemical and pharmacological properties of *Curcuma amada*: A Review. *Int. J. Res. Pharm. Sci.* 2020; **11**, 3546-55.
- [6] S Acharya, DK Patra, C Pradhan and PK Mohapatra. Anti-bacterial, anti-fungal and anti-oxidative properties of different extracts of *Bruguiera gymnorrhiza* L. (Mangrove). *Eur. J. Integr. Med.* 2020; **36**, 1-7.
- [7] G Sharma, S Dang, S Gupta and R Gabrani. Antibacterial Activity, cytotoxicity, and the mechanism of action of bacteriocin from *Bacillus subtilis* GAS101. *Med. Princ. Pract.* 2018; **27**, 186-92.
- [8] NK Kashyap, M Hait, GS Raymahapatra and MM Vaishnav. Proximate and elemental analysis of *Careya Arborea* Roxb plant's root. *ES food Agrofor. Syst.* 2022; **7**, 41-7.
- [9] M Hait and J Deepak. Physicochemical and phytochemical exploration on non-aerial part of *Curcuma amada*. *J. Pharmacogno. Phytochem.* 2018; **7**; 1306-9.
- [10] L Ying, S Ning, S Hooi-Leng, L Wei, L Yanan, C Cuicui, Z Boxin, H Xuanhe, L Zhihua and JL Yu. Antibacterial activity evaluation and mode of action study of novel thiazole-quinolinium derivatives. *RSC Adv.* 2020; **10**, 15000-14.
- [11] S Naz, S Jabeen, S Ilyas, F Manzoor, F Aslam and A Ali. Antibacterial activity of *Curcuma longa* varieties against different strains of bacteria. *Pak. J. Bot.* 2010; **42**, 455-62.
- [12] MI Rahu, SH Ahmed, NN Hussain, M Muhammad, IF Kandhro, N Lodhi, MN Pathan, I Sarker and MA Bhutto. Determination of antimicrobial and phytochemical compounds of *Jatropha curcas* plant, *Saudi J. Biol. Sci.* 2021; **28**, 2867-76.
- [13] RL McMurray, ME Ball, MM Tunney, N Corcionivoschi and C Situ. Anti-bacterial activity of four plant extracts extracted from traditional Chinese medicinal plants against *Listeria monocytogenes*, *Escherichia coli*, and *Salmonella enterica subsp. enterica serovar Enteritidis*. *Microorganisms* 2020; **8**, 1-12.
- [14] S Henciya, TS Vengateshwaran, MS Gokul, H Dahms and RA James. Antibacterial activity of Halophilic Bacteria against drug-resistant microbes associated with diabetic foot infections. *Curr. Microbiol.* 2020; **77**, 3711-23.
- [15] GS El-Baroty, HA El-Baky, RS Farag and MA Saleh. Characterization of antioxidant and antimicrobial compounds of cinnamon and ginger essential oils. *Afr. J. Biochem. Res.* 2010; **4**, 167-74.
- [16] RC Flores, M Ponzi, C Ardanaz, CE Tonn and OJ Donadel. Chemical composition of essential oil of *Baccharis salicifolia* (Ruiz and Pavon) pers. and antibacterial activity. *J. Chil. Chem. Soc.* 2009; **54**, 475-6.
- [17] MT Shaaban, MF Ghaly and SM Fahmi. Antibacterial activities of hexadecanoic acid methyl ester and green-synthesized silver nanoparticles against multidrug-resistant bacteria. *J. Basic Microbiol.* 2021; **61**, 557-68.
- [18] CD De Lacerda, JBD Souza, EV Bueno, S Medeiros, ID Cavalcanti and MF Cavalcanti. Antibacterial and anti-biofilm potential of silver nanoparticles against antibiotic-sensitive and multidrug-resistant *Pseudomonas aeruginosa* strains. *Braz. J. Microbiol.* 2021; **52**, 267-78.