Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(5); 831-835

ISSN: 0975-4873

Research Article

Burn Skin Pathogens: Isolation, Identification, Antimicrobial Activity Pattern Against *C. longa* Extract and Computational Studies of its Components

Essa Ajmi Alodeani

College of Medicine-Aldawadmi, Shaqra Univerity, Kingdom of Saudi Arabia

Available Online: 1st May, 2016

ABSTRACT

C. longa extract was obtained and tested against the burn skin pathogens such as Pseudomonas aeruginosa, Staphylococcus aureus, acinetobacter baumannii, Klebsiella spp, Proteus vulgaris and Escherichia coli. The finding of antimicrobial sxcreening disclosed that the methanol extract produced more effect than hexane extract. All the components in the extracted were examined for the drug likeness physicochemical properties, which depicted that all are in compliance with the Lipinski Rule of five except component six and all possessed bioactivity score in most active zone except component four that corresponds to moderately active zone.

Key words: *C. longa* extract, antimicrobial screening, drug likeness and physicochemical property.

INTRODUCTION

Medicinal plants has been proved as the best source for obtaining drugs and also used as traditional medicine in developed countries, as declared by WHO[1]. *Curcuma longa*, normally known as turmeric is the member of Zingiberaceae family and a perennial rhizomatous shrub[2]. It's flavouring in spicy quality and digestive ability for foods prompting its continuous use. The plant comprises the funnel shape yellow flower, oblong pointed leaves. The part rhizome, which is used as medicine followed boiling, cleaning drying and giving rise to a yellow powder[2,3]. It has been known to possess variety of biological activities antioxidant, hepatoprotective, anti-inflammatory, gastrointestinal, anticarcinogenic, cardiovascular, antimicrobial. It is also found to have potential for the treatment of *cholelithiasis, and cholestasis, hyperlipidemia, gastric ulcer, chronic anterior uveitis,* treatment of flatulence, jaundice, menstrual difficulties, hematuria, hemorrhage, and colic. The *C. longa* extract possesses desmethoxy curcumin, disdesmethoxycurcumin, curcumin, turmerone, atlantone, zingiberine, sugars, proteins, and resins. The most investigated constituent is curcumin, that occupied 0.3-5.4 percent in turmeric[4-25]. *C. longa* extraction, phytochemical screening and antimicrobial studies has been widely investigated by the researchers[16,17,22-24]. Infection is significant reason for mortality and morbidity in burn patients[38]. The rate of nosocomial infections is more in burn patients because of various factors such as nature of burn and immune system[38-40]. Our previous investigation, we also have carried out the isolation, identification of Burn skin pathogens and antimicrobial activity pattern against pyrazole derivatives[26]. It is also important to find out computationally that which component is more potent biologically active[30-37]. Keeping in mind the pharmacological potential of C. Long, and the necessity for the new anti microbial agents that can be utilized to diminish the infections caused by burn skin pathogens, we designed our investigation in such a way that deals with the extraction, antimicrobial assay and also the computational studies.

MATERIALS AND METHODS

Pathogens from Burn skin

Fifty pus samples with an average age of 10-55 years were collected, isolated maintained and preserved using 1% glycerol stock. The phenotypic characterization of the pathogens from burn skin was performed applying the procedure reported in our previous study[26].

Phytochemical extraction

The extraction C. long was achieved employing Soxhlet extraction assembly, methanol and n-hexane solvent. The Fresh rhizomes of *C. longa* were grounded and fixed inside a thimble loaded into the soxhlet extractor for 15 hr in the solvent (300 mL) with refluxing at the boiling temperature of solvent. Finally the solvent was evaporated by vacumm evaporator. Obtained extract was incubated at 40 ^{0} C for 24 hr, after that stored at 4 ^{0}C evaluation for antimicrobial screening[4-25].

Antimicrobial screening

Organism culture and in vitro screening for antimicrobial activity of *C. longa* extract against the isolated pathogens, was done by the disk diffusion method with minor modifications. The isolated and biochemically characterized pathogens: Pseudomonas aeruginosa,

*Author for Correspondence

Table 1: Representing the zone of inhibition of the methanol extract of *Curcuma longa*, against the isolated pathogens from burn patient.

	Effect of methanol extract on Microorganism				
Pathogens	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml
P. aurigenosa	24.20±0.24	18.26±0.26	28.22±0.12	12.34±0.42	10.86±0.61
S. aureus	20.12±0.22	18.32±0.62	16.64±0.36	11.30±0.72	9.82±0.45
P. vulgaris	21.16±0.32	20.36±0.43	21.44±0.35	16.64±0.45	15.32±0.54
E. coli	20.22±0.39	23.28±0.32	20.43±0.30	20.22±0.33	21.42±0.36
Klebsiella spp.	17.92±0.20	18.30±0.52	19.14±0.34	12.20±0.62	11.23±0.34
A. baumannii	13.26±0.22	17.52±0.24	16.44±0.74	10.24±0.53	8.43±0.22

Table 2: Representing the zone of inhibition the n-hexane extract of *Curcuma longa*, against the isolated pathogens from burn patient.

	Effect of n-hexane extract on Microorganism				
Pathogens	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.125 µg/ml
P. aurigenosa	20.12±0.32	15.22±0.14	13.08±0.10	12.10±0.22	10.08±0.14
S. aureus	18.34±0.32	13.64±0.20	10.16±0.42	10.12±0.18	-
P. vulgaris	15.34±0.15	11.06±0.21	10.21±0.20	-	-
E. coli	17.14±0.39	15.43±0.30	12.23±0.30	10.13±0.12	-
Klebsiella spp.	16.62±0.26	14.23±0.42	10.17±0.20	-	-
A. baumannii	11.14±0.30	10.12±0.34	-	-	-

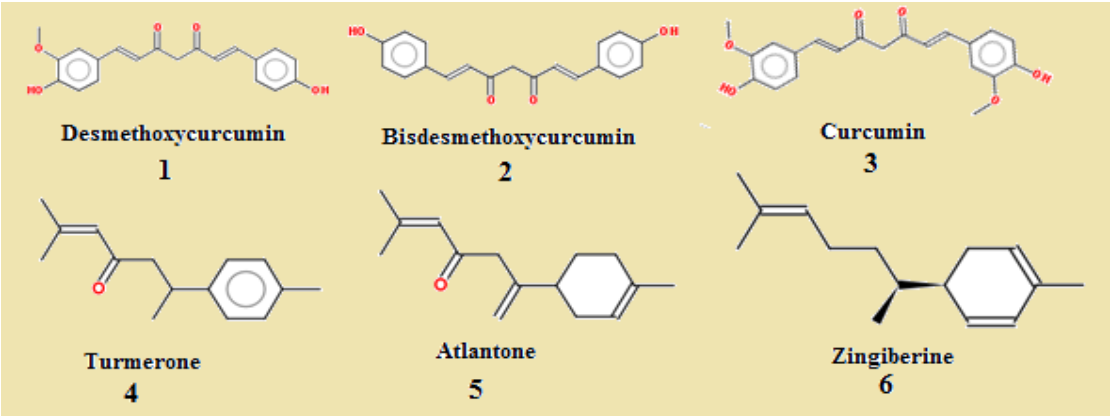

Figure 1: Exhibiting the components present in *C. longa* extract.

Table 3: Representing the zone of inhibition of the standard, methanol and hexane, against the isolated pathogens from burn patient.

Microorganism	Ciprofloxacin (10 µg/ml)	Methanol	Hexane
P. aurigenosa	34.24 ±0.31	-	-
S. aureus	21.46 ±.31	-	-
P. vulgaris	24.56±0.27	-	-
E. coli	23.82±0.47	-	-
Klebsiella spp.	21.34±0.42	-	-
A. baumannii	18.76±0.30	-	-

Staphylococcus aureus, acinetobacter baumannii, Klebsiella spp, Proteus vulgaris and Escherichia coli were subcultured in nutrient agar medium and incubated for 18 h at 37 ^{0}C. Following the incubation the bacterial cells were suspended, according to the McFarland protocol in saline solution to produce a suspension of about 10^{-5} CFU/mL. About 10 mL of this suspension was mixed with 10 mL of sterile antibiotic agar at 40 ^{0}C and poured on to an agar plate in a laminar flow cabinet. Five paper disks (6.0 mm diameter) were fixed onto nutrient agar plate. Ciprofloxacin was used as a standard drug (positive control). Methanol and hexane poured disk was used as negative control. The susceptibility of the bacteria to the test extract was determined by the formation of an inhibitory zone after 18 h of incubation at 36 ^{0}C. The zone of inhibition was calculated by antibiotic zone scale. The results were compared with the negative and positive controls[27-29].

Physicochemical properties

The drug likeness score was calculated considering partition coefficient (log P), molar refractivity, molecular weight, number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor and number of violation. All the parameters were checked with the help of software Molinspiration drug-likeness score online (www. molinspiration.com)[30-37].

Bioactivity score

The drugs are also checked for the bioactivity by calculating the activity score for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand. All the parameters were checked with the help of software Molinspiration drug-likeness score online

Table 4: Representing the physicochemical properties of all the components found in *Curcuma longa* and ciprofloxacin.

Physicochemical property score	Components						
	1	2	3	4	5	6	Standard
miLogP	2.30	2.48	2.67	4.48	4.12	5.12	-0.701
TPSA	93.07	83.83	74.60	17.07	17.07	0.00	74.569
Natoms	27	25	23	16	16	15	24.0
MW	368.38	338.36	308.33	216.32	218.34	204.36	331.347
nON	6	5	4	1	1	0	6
nOHNH	2	2	2	0	0	0	2
Nviolations	0	0	0	0	0	1	0
Nrotb	8	7	6	4	4	4	3
Volume	332.18	306.64	281.09	230.32	237.06	234.35	285.46

Table 5: Representing the bioactivity score of all the components found in *Curcuma longa* and ciprofloxacin.

Bioactivity score	Components						
	1	2	3	4	5	6	Standard
GPCR ligand	-0.06	-0.04	0.00	-0.68	-0.32	-0.39	0.12
Ion channel modulator	-0.20	-0.20	-0.14	-0.46	0.14	-0.10	-0.04
Kinase inhibitor	-0.26	-0.26	-0.26	-1.36	-1.20	-0.84	-0.07
Nuclear receptor ligand	0.12	0.18	0.25	-0.14	0.42	0.23	-0.19
Protease inhibitor	-0.14	-0.14	-0.08	-0.80	-0.49	-0.71	-0.21
Enzyme inhibitor	0.08	0.10	0.15	-0.25	0.47	0.29	0.28

(www. molinspiration.com)[30-37].

RESULTS AND DISCUSSION

Antimicrobial activity

Antibacterial assessment was done against all burn skin pathogens, subcultured in Nutrient Agar medium using ciprofloxacin as a standard drug, Methanol and n-hexane as negative control. The susceptibility of the bacteria to the tested extract was determined by the formation of an inhibitory zone after 18 h of incubation at 36 °C. The obtained results are reported in Table-1, Table-2 and Table-3, that represented that both the portion possessed antimicrobial sensitivity but the antimicrobial potential of methanol extract is more than the n-hexane extract.

Phytochemistry

Phytochemical evaluation of *C. longa*, confirmed the presence of components such as Desmethoxy curcumin, Bisdesmethoxycurcumin, curcumin, turmerone, atlantone, zingiberine[4-25], the structure for all the components are exhibited in figure-1.

Physicochemical properties

Lipinski's rule of five states that, in general, an orally active drug has not more than 5 hydrogen bond donors (OH and NH groups), not more than 10 hydrogen bond acceptors (notably N and O), molecular weight under 500 g/mol, partition coefficient log P less than 5, number of violation less than 4. All the components of C. long were found in compliance with Lipinski's rule of five except component six that exhibited the partition coefficient 5.12, the detailed results are reported in Table 4.

Bioactivity score

The bioactivity score was calculated for GPCR ligand, ion channel modulator, kinase inhibitor, nuclear receptor ligand, protease inhibitor and enzyme inhibitor. For average organic molecule the probability of bioactivity score is more than 0.00 then it is active, -0.50 to 0.0 then moderately active and if less than -0.50 then inactive. The results for bioactivity score are presented in Table 5. The results portrayed that the bioactivity score for all the components mostly lying under the zone of active drugs while the component four belongs to moderately active region.

CONCLUSION

C. longa extract was achieved and analysed for antimicrobial activity against all the pathogens isolated from burn skin. The phytochemical components of the extract were subjected for computational studies such as physicochemical and drug likeness properties. The observed results stated that methanol extract of *C. longa* possessed more antimicrobial potential than n-hexane extract, out of six only one component that is component-6 deviated from Lipinski's rule of five. All components lying under the zone for active drugs, except the component 4 that belongs to moderately activity zone for drugs.

Conflict of Interest

The authors have no conflict of interests

Acknowledgement

The authors (Dr. Mohammad Arshad & Dr. Mohammad Asrar Izhari) are thankful to Dr. Essa Ajmi Alodeani, The Dean, College of Medicine, Al-Dwadmi, Shaqra University, Kingdom of Saudi Arabia for providing facilities and support to accomplish this work.

REFERENCES

1. Aggarwal BB, Sundarma C, Malini N & Ichikawa H: Curcumin: the Indian solid gold. Adv. Exp. Med. Biol. 2007; 595:1-75.

2. Dobelis IN: Magic and Medicine of Plants. Pleasantville, NY: Reader's Digest Association, Inc. 1986.
3. Leung A: Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York, NY: John Wiley, 1980; 313-314.
4. Araújo CAC, Alegrio LV, Castro D, Lima MEF, Leon LL: Leishmania amazonensis: in vivo experiments with diarylheptanoids from Leguminosae and Zingiberaceae plants. Mem Inst Oswaldo Cruz 1998; 93 (Suppl. II): 306.
5. Bhavani Sankar TN, Murthy S: Effect of Turmeric (*Curcuma longa*) fractions on the growth of some intestinal and pathogenic bacteria in vitro. Indian J Exp Biol 1979; 17: 1363-1366.
6. Kiuchi F, Goto Y, Sugimoto N, Akao N, Kondo K, Tsuda Y: Nematocidal activity of Turmeric: synergistic action of curcuminoids. Chem Pharm Bul 1993; 41: 1640-1643.
7. Mazumber A, Raghavan K, Weinstein J, Kohn KW, Pommer Y: Inhibition of human immunodeficiency virus type-1 integrase by curcumin. 1995; Biochem Pharmacol 49: 1165-1170.
8. Wahlstrom B, Blennow G. A: study on the fate of curcumin in the rat. Acta Pharmacol Toxicol 1978; 43:86-92.
9. Ravindranath V, Chandrasekhara N: Absorption and tissue distribution of curcumin in rats. Toxicol 1980;16: 259-265.
10. Toda S, Miyase T, Arich H, et al. Natural antioxidants. Antioxidative compounds isolated from rhizome of *Curcuma longa* L. Chem Pharmacol Bull 1985; 33:1725-1728.
11. Dikshit M, Rastogi L, Shukla R, Srimal RC. Prevention of ischaemia-induced biochemical changes by curcumin and quinidine in the cat heart. Indian J Med Res 1995; 101:31-35.
12. Chandra D, Gupta S: Anti-inflammatory and anti-arthritic activity of volatile oil of *Curcuma longa* (Haldi). Indian J Med Res 1972; 60:138-142.
13. Arora R, Basu N, Kapoor V, et al: Anti-inflammatory studies on *Curcuma longa* (turmeric). Indian J Med Res 1971; 59:1289-1295.
14. Azuine M, Bhide S: Chemopreventive effect of turmeric against stomach and skin tumors induced by chemical carcinogens in Swiss mice. Nutr Cancer 1992; 17:77-83.
15. Boone CW, Steele VE, Kelloff GJ: Screening of chemopreventive (anticarcinogenic) compounds in rodents. Mut Res 1992; 267: 251-255.
16. Mari Selvam R., Athinarayanan G., Usha Raja Nanthini A., Ranjit Singh A.J.A., Kalirajan K., Mosae Selvakumar P: Extraction of natural dyes from *Curcuma longa*, Trigonella foenum graecum and Nerium oleander, plants and their application in antimicrobial fabric: Industrial Crops and Products 2015; 70: 84-90.
17. Ankur Gupta, Surabhi Mahajan, Rajendra Sharma, Evaluation of antimicrobial activity of *Curcuma longa* rhizome extract against Staphylococcus aureus, Biotechnology Reports, Volume 6, June 2015, Pages 51-55.
18. Premika G, Balasubramanian K, Kisan M. K: Molecular interactions and antimicrobial activity of curcumin (*Curcuma longa*) loaded polyacrylonitrile films. Materials Chemistry and Physics 2011; 147: 3-15.
19. Gupta A.K, Ritu M, Lal R.K: Genetic resources, diversity, characterization and utilization of agronomical traits in turmeric (*Curcuma longa* L.), Industrial Crops and Products. 2015; 77: 708-712.
20. Viviane P. Paulucci, Renê O. Couto, Cristiane C.C. Teixeira, Luis Alexandre Freitas P: Optimization of the extraction of curcumin from *Curcuma longa* rhizomes. Revista Brasileira de Farmacognosia 2013; 23 (1): 94-100.
21. Trong Tuan Dao, Phi Hung Nguyen, Ho Keun Won, Eun Hee Kim, Junsoo Park, Boo Yeon Won, Won Keun OH: Curcuminoids from *Curcuma longa* and their inhibitory activities on influenza A neuraminidases. Food Chemistry 2012; 134 (1): 1, 21-28.
22. Oluwatosin A. Adaramoye, Raline M. Anjos, Mônica M. Almeida, Robson C. Veras, Darìzy F. Silvia, Francisco A. Oliveira, Karla V. Cavalcante, Islania G. Araújo, Aldeìdia P. Oliveira, Isac A. Medeiros: Hypotensive and endothelium-independent vasorelaxant effects of methanolic extract from *Curcuma longa* L. in rats. Journal of Ethnopharmacology 2009; 124 (3) 457-462.
23. Eric WC Chan, Voon Pei Ng, Vi Vian Tan, Yin Yin Low: Antioxidant and Antibacterial Properties of Alpinia galanga, *Curcuma longa*, and Etlingera elatior (Zingiberaceae). Pharmacognosy Journal 2011; 3 (22) 54-61.
24. Jayaprakasha G.K., Jagan Mohan Rao L., Sakariah K.K: Chemistry and biological activities of *C. longa*, Trends in Food Science & Technology 2005; 16 (12) 533-548.
25. Liang-Mei Li, Jun Li, Xiu-Ying Zhang: Antimicrobial and molecular interaction studies on derivatives of curcumin against Streptococcus pneumoniaewhich caused pneumonia. Electronic Journal of Biotechnology 2016; 19: 8-14.
26. Xin Li, Shutao MA: Advances in the discovery of novel antimicrobials targeting the assembly of bacterial cell division protein FtsZ. European Journal of Medicinal Chemistry 2015; 95 (5) 1-15.
27. Alodeani E A, Arshad M, Izhari M A: Burn skin pathogens: Isolation, identification and antimicrobial activity pattern against pyrazole derivatives. American Journal of Pharm Tech research 2015, 5(6): 150-158.
28. Iram N, Khan M S, J Reshma, Arshad M, Alam M, Alam P, Khan R H, Firdaus F: Interaction mode of polycarbazole-titanium dioxide nanocomposite with DNA: Molecular docking simulation and in-vitro antimicrobial study. Journal of Photochemistry and Photobiology B: Biology 2015; 153: 20-32.
29. Bushra R, Shahadat M, Khan M A, A R., Arshad M, Rafatullah M, Naushad M: Preperation of Polyaniline

based Nanocomposite material and their Environmental Applications 2015; Int. J. Env. Sci. Technol.
30. Alodeani E A, Arshad M, Izhari M A: Anti-uropathogenic activity, drug likeness, physicochemical and molecular docking assessment of (E-)-N' -(substitutedbenzylidene)-2-(quinolin-8-yloxy) acetohydrazide. Asian Pac J Trop Biomed 2015; 5(8): 676–683.
31. Nova ulica: Molinspiration cheminformatics [homepage on the internet], SK-900 26 Slovensky Grob, Slovak Republic [cited 2012 July3], Available from http://www.molinspiration.com.
32. Verma, A: Lead finding from Phyllanthus debelis with hepatoprotective potentials Asian Pacific Journal of Tropical Biomedicine 2012; S1735-S1737.
33. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv. Drug Delivery Rev. 1997; 23(1-3): 3-25.
34. Alodeani E A, Arshad M, Izhari M A: Anti-uropathogenic activity, drug likeness, physicochemical and molecular docking assessment of (E-)-N' -(substitutedbenzylidene)-2-(quinolin-8-yloxy) acetohydrazide. Asian Pac J Trop Biomed 2015; 5(8): 676-683.
35. Alodeani E A, Arshad M, Izhari M A: Antileishmanial screening, physicochemical properties and drug likeness of pyrazole carbaldehyde derivatives: Asian Pac. J. Health Sci. 2015; 2(2): 41-47.
36. Alodeani E A, Arshad M, Izhari M A: Antileishmanial activity and computational studies of some hydrazone derivatives possessing quinoline nucleus. European Journal of Pharma and medical research. 2015; 2(7), 324-328.
37. Alodeani E A, Arshad M, Izhari M A: Drug likeness and physicochemical properties evaluation of the alkaloids found in black pepper: piperine, piperidi ne, piperettine and piperanine. European Journal of Pharma and medical research 2015; 2(6), 296-301.
38. McManus AT, Mason AD, McManus WF, Prutt BA: A decade of reduced Gram-negative infections and mortality associated with improved isolation of burned patients. Arch. Surg. 1994; 129:1306-1309.
39. Pruitt BA, McManus AT, Kim SH and Goodwin CW: Burn wound infections: current status. World J Surg. 1998; 22: 135-145.
40. Magnet M H, Arongozeb M, Khan G M, Z Ahmed: Isolation and identification of different bacteria from different types of burn wound infections and study their antimicrobial sensitivity pattern. International Journal of Research in Applied, Natural and Social Sciences. 2013; 1 (3), 125-132.
41. Church D, Elsayed S, Reid O, Winstonand B, Lindsay R: Burn Wound Infections. Clinical Microbiology Reviews 2006; 403-434.