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Burn skin pathogens: Isolation, identification and antimicrobial activity pattern against pyrazole derivatives

Essa Ajmi Alodeani[1], Mohammad Arshad[1*] Mohammad Asrar Izhari[1*]

1. *College of Medicine Al-Dawadmi, Shaqra University, Kingdom of Saudi Arabia.*

ABSTRACT

Fifty pus samples were collected from burn patients with age ranging 10-55 years with invasive wound infection using sterile cotton swabs. Pure culture were isolated, maintained, preserved and subjected for morphological characterization, accomplished by studying the colony characteristics on different culture plates followed by simple, negative and Gram staining. The cultures then also subjected for biochemical characterization performing various biochemical tests Recovered pathogens were mostly Gram negative. The most common causative agent was found to be Pseudomonas aeruginosa (39.5%), followed by Staphylococcus aureus (25.5%), Acinetobacter baumannii (10%), Klebsiella spp (4.2%), Proteus vulgaris (3.5%) and Escheritia coli (2%). All the previously synthesized compounds were tested on all the isolated pathogens using disc diffusion method and Ciprofloxacin was used as standard drug. The results revealed that out of five, three compounds (1-3) were found to possess significant antimicrobial potential against all pathogens and compounds 4, 5 were also exhibited good activity against E. coli.

Keywords: Burn skin pathogens, pyrazole, antimicrobial activity

*Corresponding Author Email: mohdarshad1985@gmail.com

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INTRODUCTION

The morbidity and mortality in hospitalized burn patients is due to infections, due to these infections the 75 % of deaths arises by thermal injury with burn in 40 % body surface area[1,2]. The serious thermal injury causes total loss of the skin surface over large areas of the body as it acts asa barrier to microbial host invasion, it is not surprising that the risk of subsequent burn wound infection and systemic infection depends on the size of the burn injury[3,4]. Due to the loss of outer layers of skin in a severe burn, there is marked loss of body fluid and danger of secondary infection with Gram negative bacteria such as *Pseudomonas* sp., or with fungi such as *Candida albicans.* Secondary infection means invasion by a new organism of tissues damaged by an earlier infection[5]. The patients with burn injury are at high risk due to the prolonged stay in hospitals, intensive diagnostic and therapeutic procedures[6]. Because of thermal injury there is a massive release of proinflammatory cytokines, chemical mediators including histamine, complement, arachidonic acid, products of the coagulation cascade, and oxygen free radicals, that produces an increase in vascular permeability leading to hypovolemia and acute renal failure[7]. By systemic inflammatory response syndrome and marked immune suppression it may be complicated. Sepsis is promoted by gastrointestinal tract because of Subsequent wound infection and bacterial translocation.[8]. A lot of research has been done on isolation, characterization and identification of the burn pathogens[9-13]. Some researchers also evaluated the sensitivity patterns of the isolated burn pathogens against a variety of anti microbial drugs[14-15]. Heterocycles, especially the five mamboed ring compounds have been long targeted due to their versatile biological applications[16-30]. On the other hand the pyrazole derivatives are reported to possess variety of biological activities[31-37]. The antimicrobial potential of pyrazole nucleus is evident from the available literature[38-42].

MATERIALS AND METHOD

Collection of clinical specimens:

Pus samples (n=50) were collected from burn patients (average age 10-55 years) with invasive wound infection using sterile cotton swabs. The most preferred locations were the upper and lower extremities.

Isolation of pure cultures using ordinary and selective media:

Specimens were inoculated aseptically in nutrient agar, mannitol salt agar, Pseudomonas agar, Mackonkey agar, Cetrimide agar, Acinetobacter medium, Eosin methylene blue agar and blood agar medium. Following inoculation all the culture plates were incubated at 37 ^{0}C for 24-48 hours in an inverted position. Pure cultures were recovered by repeated sub-culturing.

Maintenance and preservation of pure culture:

Pure cultures were maintained by periodic sub-culturing using basal medium and preserved by using 1% glycerol stock.

Phenotypic characterization of the pure cultures recovered:

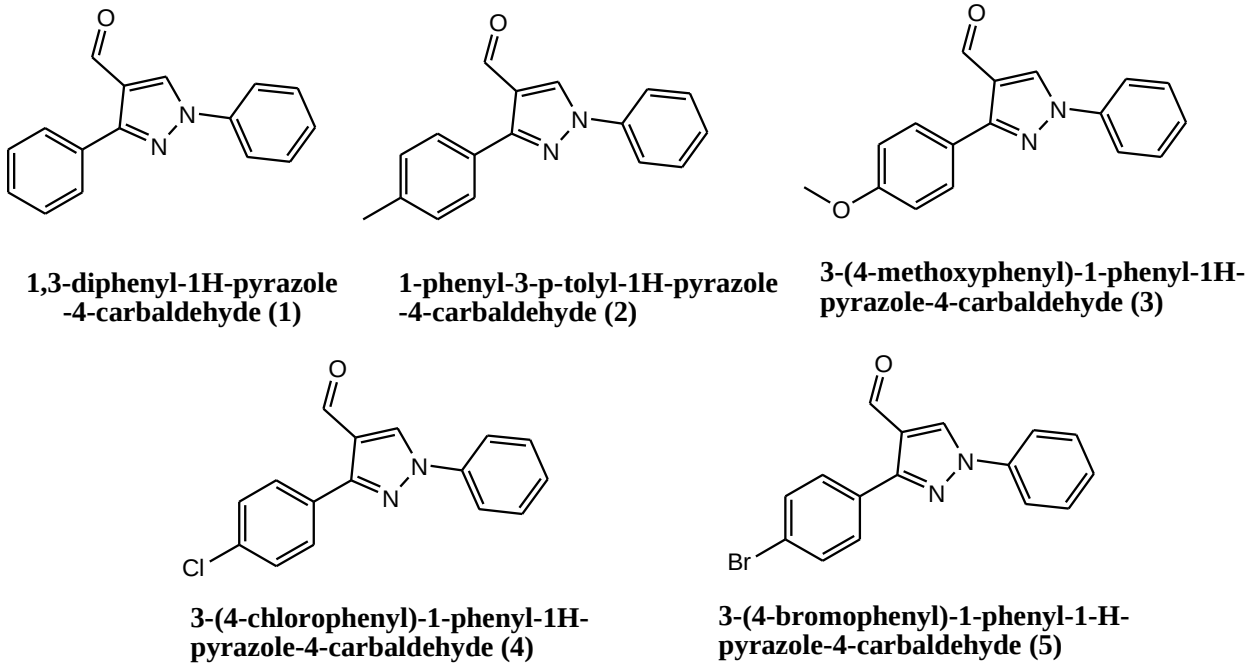
Morphological characterization was accomplished by studying colony characteristics on different culture plates followed by simple, negative and Gram staining. Biochemical characterization was carried out by performing various biochemical tests such as IMViC (Indole, Methyl red, Voges Proskauer and Citrate utilization) tests, catalase, coagulase, nitrate reduction, oxidase, acid production, H_2S production and urease tests. Hanging drop technique was used to study motility of the isolates. Cultures were named and identified based determinative bacteriology.

Chemistry:

Compounds 1-5 were synthesized according to the procedure reported in [26], and the structure of al pyrazole derivatives 1-5 are presented in **Figure-1.**

Antimicrobial activity:

Organism culture and in vitro screening for antimicrobial activity of the previously synthesized compounds against the isolated pathogens, was done by the disk diffusion method with minor



1,3-diphenyl-1H-pyrazole -4-carbaldehyde (1)

1-phenyl-3-p-tolyl-1H-pyrazole -4-carbaldehyde (2)

3-(4-methoxyphenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (3)

3-(4-chlorophenyl)-1-phenyl-1H-pyrazole-4-carbaldehyde (4)

3-(4-bromophenyl)-1-phenyl-1-H-pyrazole-4-carbaldehyde (5)

Figure-1: Representing the structures of the pyrazole derivatives 1-5.

modifications. The isolated and biochemically characterized Pseudomonas aeruginosa, Staphylococcus aureus, acinetobacter baumannii, Klebsiella spp, Proteus vulgaris, Escheritia 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. One milligram of each test compound was dissolved in 100 mL DMSO to prepare stock solution. From the stock solution different dilutions of each test compound were prepared and poured over disk plate. Ciprofloxacin was used as a standard drug (positive control). DMSO poured disk was used as negative control. The susceptibility of the bacteria to the test compounds 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.

RESULTS AND DISCUSSION

Pus samples (n=50) were collected from burn patients (average age 10-55 years) with invasive wound infection using sterile cotton swabs. The most preferred locations were the upper and lower extremities. Recovered pathogens were mostly Gram negative. The most common causative agent was found to be Pseudomonas aeruginosa (39.5%), followed by Staphylococcus aureus (25.5%), acinetobacter baumannii (10%), Klebsiella spp (4.2%), Proteus vulgaris (3.5%) and E.coli (2%) **Figure-2**. The identification of all the pathogens was done following various test such as Acid production, Urease, H_2S Production, Nitrate reduction, Oxidase, Coaggulase, catalase, citrate, Vogesproskuer, Methyl red, Indole, Motility and Morphology. The results for all the test is reported in the **Table-1.** All the previously synthesized and characterized compounds were tested on all the isolated pathogens using disc diffusion method and Ciprofloxacin was used as standard drug. The results revealed that out of five, three compounds (1-3) were found to possess significant antimicrobial potential against all pathogens and compounds 4, 5 were also exhibited good activity against E. coli. The results for antimicrobial activity are reported in **Table-2.**

Table-1: Representing the results of all tests to identify the isolated pathogens.

Tests	Pathogens					
	P. aurigenosa	S. aureus	P. vulgaris	E. coli	Klebsiella spp.	A. baumannii
Acid production	+	+	–	+	+	+
Motility	+	–	+	+	–	–
Indole	–	–	+	+	–	–
Methyl red	–	+	+	+	–	–
Vogesprosk	–	+	–	–	+	–

uer						
citrate	+	_	_	_	+	+
catalase	+	+	+	+	_	+
Coaggulase	_	+	_	_	_	_
Oxidase	+	_	_	_	_	_
Nitrate reduction	+	+	_	+	_	_
H_2S Production	_	_	+	_	_	_
Urease	+	_	+	_	+	**variable**
Morphology	**Gram negative**	**Gram positive**	**Gram negative**	**Gram negative**	**Gram negative**	**Gram negative**

Table-2: Representing the zone of inhibition of the compounds 1-5, against the isolated pathogens from burn patient.

Pathogens	**Compounds**					
	1	**2**	**3**	**4**	**5**	**Ciprofloxacin**
P. aurigenosa	24.20±0.24	18.26±0.26	28.22±0.12	12.34±0.42	10.86±0.61	34.24±0.31
S. aureus	20.12±0.22	18.32±0.62	16.64±0.36	11.30±0.72	9.82±0.45	21.46±0.36
P. vulgaris	21.16±0.32	20.36±0.43	21.44±0.35	16.64±0.45	15.32±0.54	24.56±0.27
E. coli	20.22±0.39	23.28±0.32	20.43±0.30	20.22±0.33	21.42±0.36	23.82±0.47
Klebsiella spp.	17.92±0.20	18.30±0.52	19.14±0.34	12.20±0.62	11.23±0.34	21.34±0.42
A. baumannii	13.26±0.22	17.52±0.24	16.44±0.74	10.24±0.53	8.43±0.22	18.76±0.30
DMSO	-	-	-		-	-

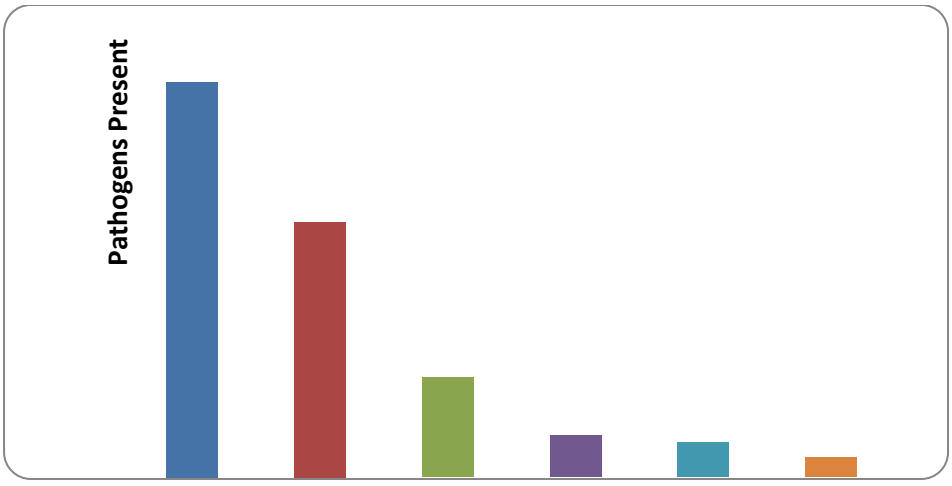


Figure-2: Representing the percentage of pathogens isolated from burn patients

CONCLUSION:

Pathogens were isolated and characterized from fifty burn patients and their antimicrobial activity pattern was evaluated using disc diffusion method and ciprofloxacin as standard. The isolated

pathogens P. aeruginosa, S. aureus, A. baumannii, Klebsiella spp, P. vulgaris, E. coli). The previously synthesized compounds 1-5 were tested against all the isolated and characterized pathogens and results revealed that out of five only three were found to possess significant antibacterial activity.

CONFLICT OF INTEREST:

The authors have no conflict of interests

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