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# In Vitro and In Vivo Evaluation of Antibacterial Activity of a Novel 2,5-Bis(Heteroamino)-1,4-Benzoquinones.

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**ABSTRACT**

A series of twenty four 2,5-Bis(heteroamino) -1,4-benzoquinones were prepared in a previous study via a reaction between hydroquinone and various heteroamine moiety, in methanol solution under microwave irradiation. Toxicity studies were carried out on the newly synthesizedaminoquinones derivatives. The compounds werescreened out, both invitroand in vivo for their ability in inhibiting various strains of bacteria ***S. aureus***, ***S. epidermidis***, and Gram-ve ***P. aeruginosa***, ***E. coli*** and correlated these activities with some QSAR (Quantitative Structure Activity Relationship) parameters. It can be concluded that the new synthesized analogues of thymoquinone have marked inhibitory effect against both Gram-postive and Gram-negative bacteria strains; in addition, it showed a powerful inhibition against ***P. aeruginosa***, a type of bacteria known to resist most antibacterial agents. The $LD_{50}$ values indicated the safety of synthesized compounds. An ointment dosage form was prepared from the compound with Vaseline (20% w/w). The antibacterial activity of the ointment tested in vivo in skin wounds infected with bacteria in rabbits. The ointment showed marked activity in treating skin wounds infected with ***S. epidermidis*** than fusidic acid (20 w/w%).It can be concluded that these new compounds have a promising antibacterial activity and may present itself as a mono therapy antibacterial agents or combined with other drugs for the treatment of infection.

**Keywords:** antibacterial, 2,5-Bis(heteroamino) -1,4-benzoquinones

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## INTRODUCTION

Thymoquinone (TQ), the principle active constituent of *Nigella sativa* (Black Cumin) posses various pharmacological effects such as immunomodulatory[1],anti- histaminic [2]; antioxidant [3-4], Anti microbial activities, which include methicillin resistance and sensitive strains of *S.aureus*[5-8]. It has also analgesic and anti- inflammatory effect [9], anti tumoractivity[10],antiangiogenesis effect.[11] The evidence for this effect is noticed since thymoquinone has been shown to decrease the number of blood vessels (antiangiogenesis effect) in the tumor mass treated with thymoquinone[12-13]. Finally, *Nigella sativa* and its active constituents, thymoquinone has been shown to have Antiproliferative effect [14].

Thymoquinone belongs to a family of quinones that can undergo redox cycling either enzymatic or non-enzymatic, thymoquinone oxidized to their corresponding semiquinone radicals to generate superoxide anion radicals which account for its biological activity [15]. Para-benzoquinones are obtained by oxidation of suitable phenolic compounds. Many of these para-benzoquinones play a vital role in electron transport in many biochemical reactions such as respiration and photosynthesis[16].

The para-benzoquinone sub-structural units are present in many natural productsfor example, thymoquinone, vitamins K1 and K2, coenzyme Q (ubiquinone), and also occur in many terpenes [17-18].

Therefore, searchingforcompounds more potent as well as lower in toxicity than thymoquinone is required. This stimulates interest in synthesizing new analogs of thymoquinone. In a recently published work by our team, a new thymoquinone analogues of 2,5-diaminoquinones incorporated with heterocyclic moietywere synthesized [19]. The principle aim of the present study, therefore, was to evaluate toxicity of the synthesized compounds, and to evaluate antibacterial activity against some clinically encountered virulent Gram positive and Gram negative bacteria and finally to evaluate antibacterial activity in vivo in infected skin wound in rabbits.

## MATERIALS AND METHODS

### General procedure for the synthesis of 2,5-bis amino-p-benzoquinone derivatives: Synthesis of Chalcones

A mixture of 0.01 mole of p- aminoacetophenone, 0.01mole of substituted benzyldehyde were dissolved in 3ml of ethanol and 0.112gm KOH then irradiated inside a microwave oven 115W for 4min, and was then transferred in to a container with crushed ice acidified with HCL.The solid was separated, filtered and recrystallized from ethanol.

### Synthesis of (pyrozole,pyrimidine) derivatives

Amixture of 0.01 mmole chalcones, 0.02 mmole of (semicarbazide, thiosemicarbazide urea, thiourea) respectively and KOH (0.02 mole; 1.12 g), were dissolved in 10 ml ethanol. The contents were thoroughly mixed. The reaction mixture was exposed to microwave irradiation in a commercially available IFB domestic microwave oven having a

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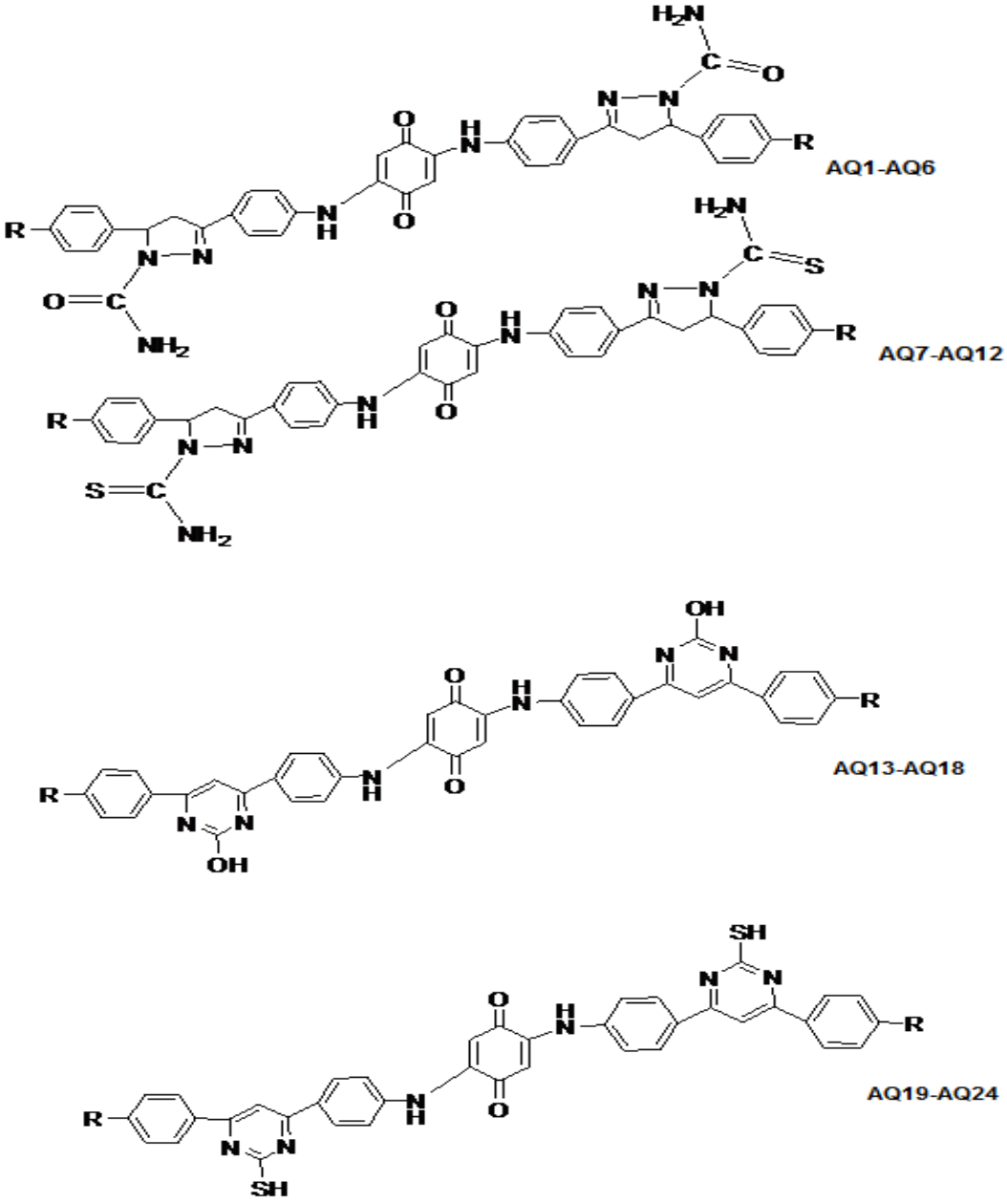
maximum power output of 480W operating at 2450Hz intermittently at 30 seconds intervals for 3-6 min on a completion of reaction as monitored by TLC. It was then cooled and transferred into a container with cold water acidified with dilute HCl. The mixture was then filtered, washed and dried. The ethanol was used to recrystallize the product.

**Synthesis of 2,5-bis amino-p-benzoquinone derivatives**

Ten milliliter of methanol solution of 2.3mmoleamine was mixed with ten milliliter of 0.05 mmole hydroquinone solution. This mixture was irradiated at (320w) for two minutes. The solution was evaporated in room temperature then the product was purified by recrystalization from methanol to obtain long crystals with different colors (red, orange, yellow). The structure of final products can be represented by the following structures:




**R: F,Cl,Br,NO,OH,$CH_3$**

**Fig (1): Chemical structure of aminoquinone derivatives**

ISSN: 0975-8585

**Determination of the median lethal dose ($LD_{50}$)of the synthesized compounds (AQ1,AQ7, AQ13 and AQ19)**

The study protocol was approved by the ethical committee of the College of Science at the University of Basrah. White albino male and female rats with average weight between 175-200 gm were obtained from the College of Veterinary Medicine, University of Basrah. They were kept in large, well ventilated cages in groups of five animals per cage with free access to food and water. Stock solution of compounds to be tested (200mg/ml)was prepared in olive oil. Five doses were chosen, two routes of administration of the compound (oral and intraperitoneal) were used for the determination of $LD_{50}$.Five groups of albino rats were randomly selected; group 1 (five rats) were given (1ml)of olive oil orally (oral controls) the animals were observed for the first 4 hrs and then at $6^{th}$, 12th $24^{th}$ and $48^{th}$hrs for signs of toxicity . The percentage of animals that had died at each dose level was transformed into Probit and then $LD_{50}$ was determined by the method of Miller and Tainter [20].

**Determination of Antimicrobial Activity**

Newly synthesized compounds were tested for their anti bacterial activity againsta series of Gram + ve bacteria such as ***S.aureus***, ***S. epidermidis***,and Gram-ve ***P.aeruginosa, E. coli***using agar – well Diffusion Method[21].

The culture strains of bacteria were maintained on nutrient agar slant at 37± 0.5°C for 24hrs. The antibacterial activity was evaluated using nutrient agar plate seeded with 0.1 ml of respective bacterial culture strain suspension prepared in sterile saline (0.85%)of 10 5CFU/ml dilutions. The wells of 5mm diameter were filled with 0.1 ml of compound solution at concentrations (7.5, 10, 15, 25)respectively. All plates were incubated at 37 ± 0.5°C for 24hrs.Zone of inhibition of the compounds in mm was recorded.

**Determination of the antibacterial activity of the compounds on bacteria-induced skin infection in rabbits**

**Preparation of animals**

Twelve white male locally breed rabbits were housed, fed and treated in accordance with the in –house guidelines for animal care. Animals were kept for two weeks for acclimatization prior to investigation. During this time, they were given lattice, dry bread, and water. Based on anti bacterial results obtained from agar well diffusion method assay, the strain of bacteria selected for this experiment was ***S.aureus***. Skin infection of rabbits with ***S.aureus*** was conducted as described by Sstpinska[22].

**Preparation of an ointment dosage form of the newly synthesized compound**

A 2% ointment of aminoquinone derivatives was prepared by mixing 0.2 gm of the newly synthesized compound AQ1 in 10 gm of white Vaseline. To ensure homogenization of the mixture, Vaseline was melted in water bath at 40°C, and by the use of spatula and stirrer

a homogenized ointment was obtained. The ointment was then kept in a clean and tight plastic container in a cool place ready for use.

**Animal's preparation**

The experiment was performed on twelve rabbits randomly divided into four groups as follow:

First group: (control group) involved clean untreated animals.
Second group: Animals to be treated with Vaseline only (not infected).
Third group: Animals to be infected by ***S. aureus*** and treated with the 0.5 gm of the 2% of prepared ointment.
Fourth group: Animals to be infected by ***S. aureus*** and treated with fusidic acid ( an ointment approved for ***S. aureus*** skin infection).

Fusidic acid ointment was used in strength of 2% formula(each 1gm of sodium fusidate contains 20 mg), manufactured by Ibn hyan –Rasheed Alfasil Company –Syria.

An area on the back of the rabbit was chosen, shaved first, sterilized with 70% alcohol then the animals, in order to minimize pain, were anesthetized with ether by inhalation before induction of infection. The area wasthen injected subcutaneously with (0.05$cm^3$ of bacterial suspension, containing 1011 cell/ $cm^3$, while first and second groups were injected with 0.05 cm3 of normal saline.

## RESULTS

The $LD_{50}$ values of the orally administered synthesized compounds in rats were found to be (4.645, 4.677, 4.59, 4.67gm/kg) respectively. These results are presented in tables (1-4).

**Table (1): $LD_{50}$ of orally administered newly synthesized compound (AQ1)**

| Con. | Number of Rats | Dose g/kg | Number of dead Rats | | | | Total dead Rats | % of dead Rats | $LD_{50}$ g/kg |
|---|---|---|---|---|---|---|---|---|---|
| | | | 6 hrs | 12 hrs | 24 hrs | 48 hrs | | | |
| (AQ1)1 | 5 | 3.5 | 0 | 0 | 1 | 0 | 1 | 20 | 4.645 |
| (AQ1)2 | 5 | 4.5 | 0 | 1 | 1 | 0 | 2 | 40 | |
| (AQ1)3 | 5 | 5.5 | 0 | 1 | 2 | 0 | 3 | 60 | |
| (AQ1)4 | 5 | 6.5 | 2 | 1 | 2 | 0 | 5 | 100 | |

ISSN: 0975-8585

**Table (2): $LD_{50}$ of orally administered newly synthesized compound (AQ7)**

| Con. | Number of Rats | Dose g/kg | Number of dead Rats | | | | Total dead Rats | % of dead Rats | $LD_{50}$ g/kg |
|---|---|---|---|---|---|---|---|---|---|
| | | | 6 hrs | 12 hrs | 24 hrs | 48 hrs | | | |
| (AQ7)1 | 5 | 3.5 | 0 | 0 | 1 | 0 | 1 | 20 | 4.677 |
| (AQ7)2 | 5 | 4.5 | 0 | 1 | 2 | 0 | 3 | 60 | |
| (AQ7)3 | 5 | 5.5 | 0 | 3 | 0 | 0 | 3 | 60 | |
| (AQ7)4 | 5 | 6.5 | 3 | 2 | 0 | 0 | 5 | 100 | |

**Table (3): $LD_{50}$ of orally administered newly synthesized compound (AQ13)**

| Con. | Number of Rats | Dose g/kg | Number of dead Rats | | | | Total dead Rats | % of dead Rats | $LD_{50}$ g/kg |
|---|---|---|---|---|---|---|---|---|---|
| | | | 6 hrs | 12 hrs | 24 hrs | 48 hrs | | | |
| (AQ13)1 | 5 | 3.5 | 2 | 0 | 0 | 0 | 2 | 20 | 4.59 |
| (AQ13)2 | 5 | 4.5 | 2 | 1 | 0 | 0 | 3 | 60 | |
| (AQ13)3 | 5 | 5.5 | 2 | 0 | 1 | 1 | 4 | 80 | |
| (AQ13)4 | 5 | 6.5 | 3 | 0 | 1 | 1 | 5 | 100 | |

**Table (4): $LD_{50}$ of orally administered newly synthesized compound (AQ19)**

| Con. | Number of Rats | Dose g/kg | Number of dead Rats | | | | Total dead Rats | % of dead Rats | $LD_{50}$ g/kg |
|---|---|---|---|---|---|---|---|---|---|
| | | | 6 hrs | 12 hrs | 24 hrs | 48 hrs | | | |
| (AQ19)1 | 5 | 3.5 | 0 | 2 | 0 | 0 | 2 | 20 | 4.67 |
| (AQ19)2 | 5 | 4.5 | 1 | 1 | 0 | 0 | 2 | 20 | |
| (AQ19)3 | 5 | 5.5 | 3 | 1 | 0 | 0 | 4 | 80 | |
| (AQ19)4 | 5 | 6.5 | 2 | 3 | 0 | 0 | 5 | 100 | |

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The antibacterial assay in vitro revealed that the synthesized compounds had a potent selective activity against gram positive bacteria while some of them showed activity against gram positive as well as gram negatives. Table (5) displays antibacterial activity against four strains of bacteria with four concentrations.

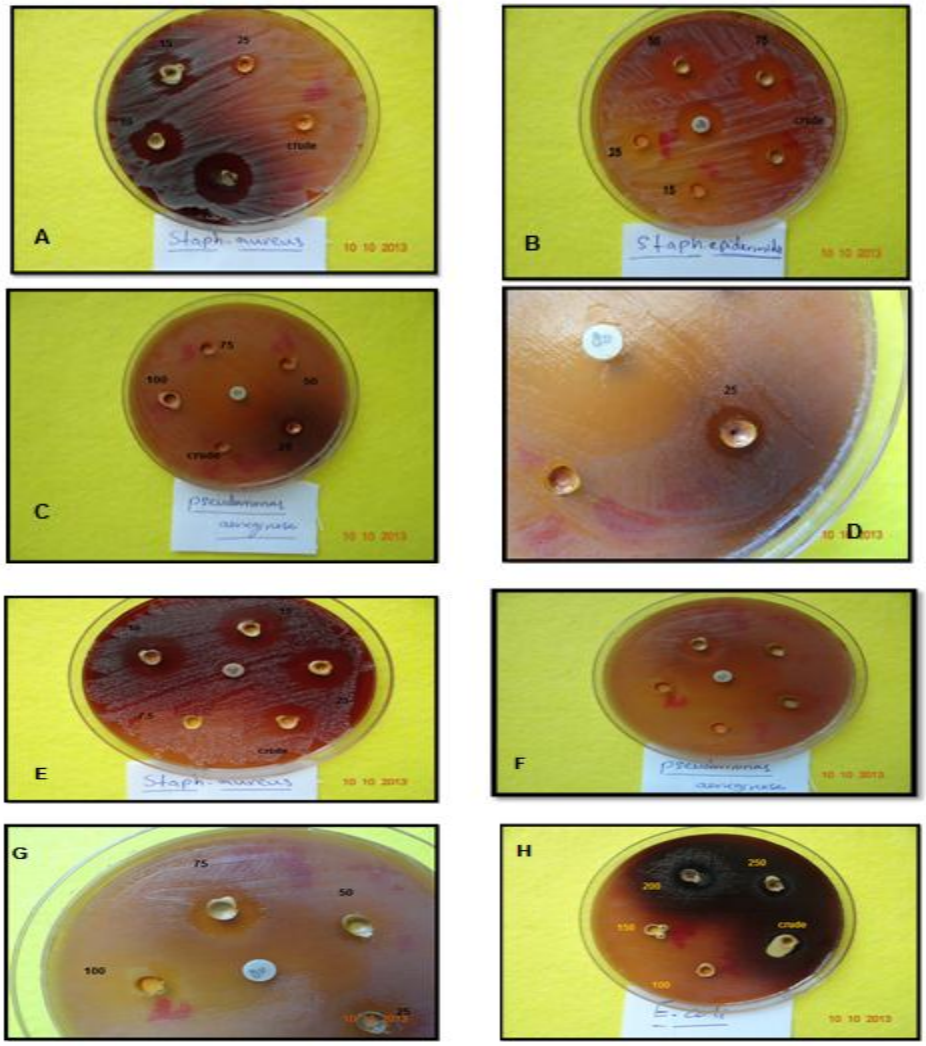


**Fig (2): A: Inhibition Zone of AQ13 against *Staphylococcus aureus* at Concentrations (7.5, 10, 15, 25) µg/ml**
**B and C: Inhibition Zone Of AQ13 against *Pseudomonas aeruginosa* at Concentrations (25,50,75 100 ) µg/ml**
**D and E: Inhibition Zone Of AQ1 against *Staph. epidermids, Staphylococcus aureus* at Concentrations (15,25,50,75 ),(7.5,10,15,25) respectively µg/ml**
**F and G: Inhibition Zone Of AQ1 against *Pseudomonas aeruginosa* at Concentrations (25,50,75,100 ) µg/ml**
**H: Inhibition Zone Of AQ1 against *E. coli* at Concentrations ( 100,150,200,250 ) µg/ml**

ISSN: 0975-8585

**Table (5): The effect of concentrations of newly synthesized compounds on inhibition zone (mm) against Bacteria strains**

| Germ | *S. aureus* | | | | *S. e.* | | | | *Ps.* | | | | *E. coli* | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Symbol / Conc. µg /ml | 7.5 | 10 | 15 | 25 | 15 | 25 | 50 | 75 | 25 | 50 | 75 | 100 | 100 | 150 | 200 | 250 |
| AQ1 | 5 | 15 | 24 | 29 | 10 | 12 | 25 | 29 | 22 | 0 | 1 | 1 | 5 | 12 | 20 | 15 |
| AQ2 | 10 | 12 | 13 | 14 | 17 | 17 | 19 | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| AQ3 | 5 | 8 | 13 | 15 | 10 | 10 | 13 | 15 | 0 | 0 | 5 | 7 | 0 | 4 | 8 | 10 |
| AQ4 | 0 | 0 | 2 | 2 | 0 | 1 | 1 | 2 | 1 | 1 | 2 | 2 | 0 | 0 | 1 | 1 |
| AQ5 | 7 | 10 | 10 | 11 | 8 | 10 | 14 | 15 | 5 | 8 | 10 | 12 | 0 | 0 | 0 | 5 |
| AQ6 | 1 | 1 | 6 | 10 | 1 | 1 | 2 | 2 | 6 | 8 | 13 | 19 | 0 | 0 | 0 | 0 |
| AQ7 | 4 | 8 | 10 | 11 | 4 | 6 | 7 | 9 | 5 | 8 | 9 | 10 | 0 | 0 | 0 | 0 |
| AQ8 | 7 | 7 | 10 | 10 | 0 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 0 | 0 | 0 |
| AQ9 | 10 | 14 | 15 | 19 | 15 | 20 | 23 | 25 | 11 | 13 | 14 | 15 | 0 | 0 | 5 | 10 |
| AQ10 | 7 | 9 | 12 | 25 | 1 | 2 | 4 | 18 | 1 | 2 | 4 | 1 | 4 | 4 | 4 | 10 |
| AQ11 | 2 | 3 | 10 | 15 | 7 | 8 | 15 | 18 | 0 | 8 | 8 | 10 | 1 | 3 | 7 | 7 |
| AQ12 | 8 | 8 | 10 | 12 | 8 | 10 | 17 | 18 | 1 | 1 | 1 | 6 | 0 | 0 | 0 | 0 |
| AQ13 | 34 | 20 | 15 | 8 | 17 | 15 | 6 | 4 | 4 | 4 | 3 | 1 | 4 | 3 | 1 | 1 |
| AQ14 | 14 | 13 | 12 | 6 | 2 | 19 | 18 | 6 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| AQ15 | 11 | 8 | 8 | 5 | 13 | 11 | 8 | 5 | 3 | 3 | 1 | 1 | 1 | 1 | 0 | 0 |
| AQ16 | 13 | 14 | 8 | 4 | 20 | 18 | 17 | 6 | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| AQ17 | 21 | 20 | 19 | 8 | 11 | 8 | 5 | 5 | 5 | 5 | 4 | 1 | 2 | 2 | 1 | 0 |
| AQ18 | 25 | 14 | 12 | 4 | 14 | 13 | 10 | 5 | 6 | 4 | 1 | 0 | 7 | 2 | 1 | 0 |
| AQ19 | 20 | 18 | 18 | 10 | 11 | 9 | 5 | 1 | 8 | 5 | 1 | 1 | 1 | 0 | 0 | 0 |
| AQ20 | 19 | 12 | 12 | 8 | 13 | 3 | 2 | 2 | 1 | 5 | 4 | 1 | 2 | 2 | 1 | 0 |
| AQ21 | 10 | 9 | 3 | 3 | 8 | 4 | 4 | 2 | 12 | 11 | 6 | 5 | 2 | 2 | 1 | 0 |
| AQ22 | 10 | 10 | 7 | 7 | 13 | 10 | 10 | 8 | 11 | 10 | 8 | 5 | 5 | 1 | 0 | 0 |
| AQ23 | 8 | 7 | 7 | 2 | 6 | 2 | 1 | 1 | 12 | 8 | 2 | 2 | 2 | 2 | 1 | 1 |
| AQ24 | 15 | 10 | 8 | 8 | 4 | 2 | 2 | 1 | 7 | 2 | 0 | 0 | 2 | 2 | 1 | 1 |

**Table (5): The values of MIC (µg / ml )& C log p for compounds ( AQ13-AQ24) against *S. aureus***

| Substituent (R) | Pyrimidin-2-thiol (AQ19-AQ24) | | Pyrimidin-2-ol (AQ13-AQ18) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 50 | 10.611 | 25 | 9.859 |
| Cl | 50 | 11.751 | 25 | 10.999 |
| Br | 25 | 12.051 | 15 | 11.299 |
| $NO_2$ | 75 | 9.824 | 50 | 9.081 |
| $CH_3$ | 15 | 11.311 | 10 | 10.552 |
| OH | 25 | 9.495 | 15 | 8.735 |

**Table (6): The values of MIC (µg / ml )& C log p for compounds ( AQ13-AQ24) against *S. epidermidis***

| Substituent (R) | Pyrimidin-2-thiol (AQ19-AQ24) | | Pyrimidin-2-ol (AQ13-AQ18) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 15 | 10.611 | 15 | 9.859 |
| Cl | 15 | 11.751 | 15 | 10.999 |
| Br | 15 | 12.051 | 15 | 11.299 |
| $NO_2$ | 20 | 9.824 | 20 | 9.081 |
| $CH_3$ | 15 | 11.311 | 15 | 10.552 |
| OH | 20 | 9.495 | 20 | 8.735 |

ISSN: 0975-8585

**Table (7): The values of MIC (µg / ml )& C log p for compounds ( AQ13-AQ24) against *P. aeruginosa***

| Substituent (R) | Pyrimidin-2-thiol (AQ19-AQ24) | | Pyrimidin-2-ol (AQ13-AQ18) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 2.5 | 10.611 | 2.5 | 9.859 |
| Cl | 2.5 | 11.751 | 2.5 | 10.999 |
| Br | 2.5 | 12.051 | 2.5 | 11.299 |
| $NO_2$ | 5 | 9.824 | 5 | 9.081 |
| $CH_3$ | 2.5 | 11.311 | 2.5 | 10.552 |
| OH | 5 | 9.495 | 5 | 8.735 |

**Table (8): The values of MIC (µg / ml )& C log p for compounds ( AQ13-AQ24) against *E. coli***

| Substituent (R) | Pyrimidin-2-thiol ( AQ19-AQ24) | | Pyrimidin-2-ol ( AQ13-AQ18) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 75 | 10.611 | 75 | 9.859 |
| Cl | 50 | 11.751 | 50 | 10.999 |
| Br | 50 | 12.051 | 50 | 11.299 |
| $NO_2$ | 100 | 9.824 | 100 | 9.081 |
| $CH_3$ | 25 | 11.311 | 50 | 10.552 |
| OH | 100 | 9.495 | 100 | 8.735 |

**Table (9): The values of MIC (µg / ml )& C log p for compounds (AQ1-AQ12) against *S. aureus***

| Substituent (R) | Thio semi Pyrazole (AQ7-AQ12) | | Semi Pyrazole (AQ1-AQ6) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 10 | 5.726 | 10 | 5.726 |
| Cl | 15 | 6.866 | 15 | 6.866 |
| Br | 7.5 | 7.166 | 7.5 | 7.166 |
| $NO_2$ | 25 | 4.926 | 25 | 4.926 |
| $CH_3$ | 15 | 6.438 | 15 | 6.438 |
| OH | 25 | 4.106 | 25 | 4.106 |

**Table (10): The values of MIC (µg / ml )& C log p for compounds (AQ1-AQ12) against *S. epidermidis***

| Substituent (R) | Thio semi Pyrazole (AQ7-AQ12) | | Semi Pyrazole (AQ1-AQ6) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 50 | 5.726 | 25 | 5.726 |
| Cl | 50 | 6.866 | 25 | 6.866 |
| Br | 25 | 7.166 | 15 | 7.166 |
| $NO_2$ | 75 | 4.926 | 50 | 4.926 |
| $CH_3$ | 15 | 6.438 | 10 | 6.438 |
| OH | 25 | 4.106 | 15 | 4.106 |

**Table (11): The values of MIC (µg / ml )& C log p for compounds (AQ1-AQ12) against *P. aeruginosa***

| Substituent (R) | Thio semi Pyrazole (AQ7-AQ12) | | Semi Pyrazole (AQ1-AQ6) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 75 | 5.726 | 75 | 5.726 |
| Cl | 50 | 6.866 | 50 | 6.866 |
| Br | 50 | 7.166 | 50 | 7.166 |
| $NO_2$ | 100 | 4.926 | 100 | 4.926 |
| $CH_3$ | 50 | 6.438 | 50 | 6.438 |
| OH | 100 | 4.106 | 100 | 4.106 |

**Table (12): The values of MIC (µg / ml )& C log p for compounds (AQ1-AQ12) against *E. coli***

| Substituent (R) | Thio semi Pyrazole (AQ7-AQ12) | | Semi Pyrazole (AQ1-AQ6) | |
|---|---|---|---|---|
| | MIC | C log P | MIC | C log P |
| F | 100 | 5.726 | 100 | 5.726 |
| Cl | 100 | 6.866 | 100 | 6.866 |
| Br | 75 | 7.166 | 50 | 7.166 |
| $NO_2$ | 100 | 4.926 | 100 | 4.926 |
| $CH_3$ | 75 | 6.438 | 75 | 6.438 |
| OH | 100 | 4.106 | 100 | 4.106 |

ISSN: 0975-8585


**Fig(3): a,b,c and d are for fucidic acid ointment treatment: (a) skin after two days of Infection , (b): After four days of Infection , (c): one day of treatment with ( Fusidic acid) , (d): three days treatment with ( Fusidic acid) . Photos e,f,g,h are for treatment with ointment prepared from the newly synthesized compound : ( e) skin after two days of Infection ,(f): After four days of Infection, (g): one day of treatment with the compound , (h): three days of treatment with the compound.**

## DISCUSSION

$LD_{50}$ of the tested compounds after oral administration in rats were (4.645,4.677,4.59,4.67gm/kg) respectively, which were comparable to previously reported values of ( 2.4gm/kg )[23] for thymoquinone which is a quinoned compound has a 1,4-benzoquinone moiety. According to the chemical toxicity classification, chemical compounds are considered moderately toxic if the probable oral lethal dose for humans ranging between (0.5-5gm/kg). The toxicity of the synthesized aminoquinone derivatives compounds in the present study are considered relatively safe after oral administration.

The antibacterial activity of aminoquinone derivatives were tested against four types of bacteria with diverse properties; two of them were Gram positive and two Gram negative. The observed activity could indicate selective inhibition of these bacteria and may be attributed to substituent's group linked to the parent compound, physiochemical properties and nature of the bacteria species[24].The contrasts in biological activities against the studied bacteria is attributable to the permeability barrier to compounds since the structure properties of cell membrane between bacterial cells are different such as the presence of outer membrane of peptidoglycon in E. coli may prevent the transport of compounds into the cell[25].

Among the studied compounds, AQ1was found more potent against ***S. aureus***, witha reverse correlation between concentration and inhibition zone. This can be explained by means of hydrogen bonding formation between molecules as the chemical structures of compounds (AQ13- AQ24),which can form inter molecular hydrogen bonding, may induce the molecules to form aggregation and resulted in decreasing the number of free molecules which can impose an inverse affect on biological activity and inhibition zone[26-27].Physiochemical property Clog p (index of hydrophopicity, which is a characteristic for a chemical structure and substituents of compound)was calculated using a special program, Hyperchem 10.0. The structures were geometry optimized for all compounds by using PM3 method, from tables (2,3,4). It was observed that as hydrophopicity value increased, MIC was reduced [28].

The newly synthesized compounds demonstrated marked activity in clearing skin infection caused by microorganisms which are described as resistant to a wide range of antibacterial agents. The time required for effect to appear, is shorter with the use of dosage form prepared from the synthesized compound compared with the effect of a standard anti bacterial agents like (fucidic acid) which is frequently prescribed for skin infection with S. Auereus type of bacteria. These results may suggest that the newly synthesized amino quinones derivatives are potent agents in treating bacterial infection.

## CONCLUSION

The 2,5-Bis (Pyrimidine amino) -1,4-benzoquinone derivatives, were synthesized successfully throughout aerobic oxidation of hydroquinone followed by conjugate addition of primary amine(pyrimidine amino moiety) according to Michael addition protocol under microwave irradiation. All spectral data confirmed the full agreement of the chemical structure for synthesized compounds. These compounds revealedgood activity against Gram positive bacteria and the compound AQ14 was superior in inhibiting bacterial growth compared with other synthesized compounds. The antibacterial assay in vivo as well as the low toxicity of these compounds may put them as a candidate for a promising antibacterial agents.

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