Evaluation of the antimicrobial activity, sub-chronic toxicity and wound healing effect of Cunninghamellaelegans extract and its Isolated Compounds

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Abstract:Aim: Evaluation of the *in-vitro* antimicrobial and *in-vivo* wound healing activities of the total alcohol extract and isolated compounds from *Cunninghamellaelegans(C. elegans*). **Methods**: Different G-ve and G+ve bacteria were used in the antimicrobial study, and the excision wound model in guinea pigs was used. Identification of compounds was done using different spectroscopic methods. **Results and Conclusion**: *C. elegans* total extract, ethyl acetate and ether successive extracts showed high activity against *Staphylococcus aureus.* Three fatty acids were isolated and identified as Palmitic acid **1**, Oleic acid **2** and Stearic acid **3**in addition to seven compounds; α-amyrin**4**.β–sitosterol**5**, 2- (6-amino-9H-purin-9-yl) - 5- (hydroxymethyl) oxolane- 3, 4- diol (adenosine) **6**, Uridine (pyrimidine 2,4-dione,1-ribose) **7**, Pyrimidine-2, 4-dione (Uracil) **8**, 3,4,5-trihydroxy-benzoic acid (Gallic acid) **9**, and 3-(methoxycarbonyl) but-3-enoic acid **10** were isolated from *C. elegans* extract. Among these compounds, adenosine was the most active antimicrobial compound *in-vitro* against *Staphylococcus aureus*. The topical application of the ethanol extract of *C. elegans*(5 mg mL^{-1}) and adenosine (1 mg mL^{-1}) produced complete wound healing activity in experimentally infected guinea pigs after 18 days. The total ethanol extract of the investigated fungus was safe up to 5000 mg kg^{-1} and did not produce any significant change in liver and kidney functions after oral administration (200 mg kg^{-1}) for 35 consecutive days.
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Key words: Adenosine, *Cunninghamellaelegans*, *Staphylococcus aureus.* Antimicrobial activity, liver and kidney function, wound healing.

1.Introduction:

The fungal kingdom includes many species with unique and unusual biochemical pathways. The products of these pathways include important pharmaceuticals such as penicillin, cyclosporine and statins potent poisons, such as aflatoxins and trichothecenes; and some Janus-faced metabolites that are both toxic and pharmaceutically useful, such as the ergot alkaloids. All of theseproducts, along withother natural low-molecular-weight fungal metabolites, are classified as secondary metabolites **(Keller *et al.*, 2005**). These fungalcompounds have a wide range of chemical structure and biological activities; they are derived from many different intermediates by special enzymatic pathways encoded by specific genus (**Deacon, 1997).** The fungal secondary metabolites, or biochemical indicators of fungal development, are of intense interest to humans (**Bok and Keller, 2004).**

Cunninghamella is a genus of fungus in the Mucorales order, and the family Cunninghamellaceae found in soil and plant material, particularly at Mediterranean and subtropical zones. Members of this genus are often used in drug metabolic studies,as they metabolize a wide range of drugs in manners similar to mammalian enzyme systems **(Asha and Vidyavathi, 2009)**. Many species are also capable of oxidizing polycyclic aromatic hydrocarbons.

Based on the aforementioned facts and as a continuation of our previous efforts the aim of the present study is to evaluate the antimicrobial and wound healing activity of three extracts obtained from *Cunninghamellaelegans* and to isolate the most effective compounds. Such approach may upgrade these compounds toward biotechnological application.

2. Material and Methods

Fungal Material

The investigated fungi, *Cunninghamellaelegans*Lendner (DSM 1908) were obtained from DSMZ(German Collection of

Microorganisms and Cell Cultures**).** The direct inoculation method was used for sampling and isolation of fungal isolates.

Extraction and isolation

The mycelia mat of *C. elegans* (700 g) was harvested, washed with distilled water, then extracted by refluxing in boiled methanol (2 liter) for 2 hours and filtered off. The mycelia residue was re-extracted again for three times. The combined filtrates were concentrated under reduced pressure at temperature not exceeding 35˚C. The obtained residue (67 g) was kept for investigation and symbolized as R1. The growth medium (4 L) was extracted by n-butanol (8 L). This step was repeated until complete extraction. The butanol extract was filtered on anhydrous sodium sulphate. The combined filtrates were subjected to concentration using reduced pressure at temperature not exceeding 35˚C. The obtained residue (60 g) was kept for investigation.

The obtained residue of mycelia mat extract (60 g) was diluted with distilled water (200 ml) and successively extracted using diethyl ether, chloroform, ethyl acetate and n-butanol. Each successive extract was dried over anhydrous sodium sulphate and concentrated using reduced pressure to obtained residues of 6, 10, 20 and 35 g, respectively**.** All fungal successive extracts were chromatographically investigated on pre-coated silica gel GF plates using (Chloroform- diethyl ether 50:50 v/v) **a**, (Chloroform-methanol 96:4 v/v) **b**, and (Ethyl acetate- Methanol-water30: 5: 4v/v/v) **c** as solvents systems. Both diethyl ether and chloroform extracts showed the same pattern on TLC (Numbers and color of spots) on the other hand both ethyl acetate and n-butanol extracts were the similar to each other therfor each similar extracts were collected together and symbolizes as **A1** and **A2**. Isolation of the active compounds was carried out using from extracts **A1** and **A2**separately (using system a, b & c). Accordingly, 15 g of **A1**was applied on column (5 x 120cm) backed with silica gel (450 g) and eluted with ether gradually increased with chloroform. Hundred fractions(50 ml, each) were collected and reduced into 5 mean fractions (according to number, colure and R_f of the spots). Each fraction was concentrated under reduced pressure to yield (0.9, 3.0, 5.0,2.1 and 1.1 g, respectively). The residue of each fraction group was separately reapplied on columns backed with Silica gel (30, 60, 100, 50 and 30 g respectively) and eluted with $CHCl_3$ gradually increased with methanol, from which compounds **1-5** were isolated in semi purified condition, therefore they purified by preparative TLC and re-purified by applying on column packed with sephadex LH 20 and eluted with methanol-water 1:1 v\v. Extract **A2 (20 g)** was applied on column (5 x 150cm) backed with silica gel (600g) and eluted with ethyl acetategradually increased with methanol. Ninety fractions(100 ml, each) were collected and reduced into 3 mean fractions (according to number, colure and R_f of the spots). Each fraction was concentrated under reduced pressure to yield (3.1, 2.9 and 4.1 g, respectively). The residue of each fraction group was separately reapplied on columns backed with Silica gel (85, 70 and 120 g respectively) and eluted with ethyl acetate gradually increased with methanol, from which compounds **6-10** were isolated.

Compound 1: This compound obtained as yellowish residue (400mg) with R_f0.61in (system d), m.p.(13˚-14˚). By comparing the obtainedspectroscopic data analysis(^{1}H-NMR.^{13}C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Rao*et al*., 2000)** this compound identified as; (9*Z*)-Octadec-9-enoic acid (Oleic acid).

Compound 2: Obtained as white residue (300mg) with R_f 0.55 in (system d), m.p. (62.9 °C) b. p. (351-352 °C). By comparing the obtainedspectroscopic data analysis(^{1}H-NMR.^{13}C-NMR and DEPT COSY, HSQC and HMBC) with published one (**Amade***et al*., 1994) this compound identified as; Palmitic acid.

Compound 3: this compound obtained as white needle crystals (104mg), with R_f 0.52 (in system d), (m.p. 69.6˚- 72˚). By comparing the obtained spectroscopic data analysis(^{1}H-NMR.^{13}C-NMR and DEPT COSY, HSQC and HMBC) with published one (**Rao*et al*., 2000**) this compound identified as; stearic acid.

Compound 4: Compound obtained as white needle crystals from methanol (100 mg), R_f (in system c) was 0.62, (m.p 185˚-187˚). By comparing the obtainedspectroscopic data analysis(^{1}H-NMR.^{13}C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Holzgrabe et al., 2008)** this compound identified as;α-amyrin.

Compound 5: It is white needle crystals (150 mg),R_f (in system c) was 0.44, (m.p. 137˚-139˚). By comparing the obtainedspectroscopic data analysis(^{1}H-NMR.^{13}C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Kambojand Saluja2011**) this compound identified as; as β–sitosterol

Compound 6: Obtained as white residue (20 mg) with R_f 0.12 in (system e), m.p (233 °C).By comparing the obtainedspectroscopic data analysis(1H-NMR.13C-NMR and DEPT COSY, HSQC and HMBC) with published one (Williams and Ian, 1994).this compound identified as; 2-(6-amino-9*H*-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol (adenosine).

Compound 7: Obtained as yellow residue (mg) with R_f 0.16 in (system a), m.p(°C). By comparing the obtainedspectroscopic data analysis(1H-NMR.13C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Williams and Ian, 1994)**.this compound identified as Uridine (pyrimidine 2,4-dione,1-ribose).

Compound 8: Obtained as yellow residue (mg) with R_f 0.34 in (system a), m.p (335 °C). By comparing the obtained spectroscopic data analysis(1H-NMR.13C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Williamsand Ian1994)**.this compound identified as Pyrimidine-2,4-dione (Uracil) (Silverstein *et al*., 1984).

Compound 9: Obtained as yellow orange residue (mg) soluble in methanol, with R_f 0.72 in (system a), m.p (252 °C). By comparing the obtainedspectroscopic data analysis(1H-NMR.13C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Williams and Ian, 1994).**this compound identified as 3,4,5-trihydroxy-benzoic acid (Gallic acid).

Compound 10: Compound **SA_5** (mg), colorless crystals from methanol, m.p (66-68°C),withR_f 0.21 in (system c). By comparing the obtainedspectroscopic data analysis(1H-NMR.13C-NMR and DEPT COSY, HSQC and HMBC) with published one **(Williams and Ian, 1994).**This compound identified as; 3-(methoxycarbonyl) but-3-enoic acid.

Biological Studies:

1) Antimicrobial Activity

Preparation of the extracts:

The alcohol extract of both extra and intra cellular of*C. elegans* was used for testing their antimicrobial activity. The extract (0.5gm each) were dissolved in 10ml water separately. Same concentration of the fractions were prepared (ether, petroleum ether, ethyl acetate, chloroform, butanol).

Test organisms

Representatives of Gram-negative bacteria; namely, *Salmonella typhimuium* (RCMB 0104), *Escherichia coli* (RCMB 0103), *Pseudomonas aeruginosa* (RCMB 0102) and Gram- positive bacteria; namely, *Bacillus subtilis* (RCMB 0107), *Staphylococcus aureus*(RCMB 0106), *Streptococcus pyogenes* (RCMB 0109(, unicellular fungi; namely, *Candida albicans* (RCMB 5002), and filamentous fungi; namely, *Aspergillusfumigatus* (RCMB 2003) and *Penicilliumexpansum* (RCMB 1006) were used as test organisms.

Antimicrobial screening

The disc diffusion and agar well diffusion methods (Duguid*et al*., 1978)were used for the antimicrobial screening and determination of minimum inhibitory concentration (MIC), for the extract of *C.elegans*.

2) Pharmacological Activities:

Preparation of fungal extract: The ethanol extracts of *C. elegans*was suspended individually in sterile normal saline (0.9 % NaCl) with the aid of few drops of tween 80 immediately before use.

Animals: Swiss albino mice (20–25 g), male Sprague–Dawley rats (160–180 g) and male guinea pigs (240-260 g) were supplied by the animal house of King Saud University, Riyadh, KSA. The animals were left for 2 weeks at standard laboratory conditions before use and maintained on standard pellet diet and water *ad libitum* throughout the experiment.

A. Acute Toxicity: Mice were divided into groups each of 6. The ethanol extract of *C. elegans*was administered orally in increasing doses (up to 5000 mg kg^{-1}). After 24 h, the LD_{50} was calculated for each extract according to **Ghosh (1984).**

B. Sub-chronic Toxicity: Ten rats were randomly divided into 2 equal groups, received orally the vehicle (5 ml $kg^{-1}$of 1% v/v Tween 80), the ethanol extract of *C. elegans*(200 mg kg^{-1}) for 35 consecutive days **(Rong*et al*., 2009)**. At the end of the experimental period, blood samples were collected from the retro-orbital venous plexus of each rat, sera were separated and used for measurement of liver and kidney markers. The levels of ALT and AST **(Reitman and Frankel, 1957)**, alkaline phosphatase (ALP) **(Babson *et al*, 1966),** total protein (TP) **(Henary*et al*, 1974),** albumin (Alb**) (Doumas*et al*, 1971),** total bilirubin (TB) **(Walter &Gerarade, 1970)**, were determined in serum to evaluate liver functions in rats.Serum concentrations of urea **(Walter andGerarade , 1970)** and creatinine **(Kroll *et al.*, 1987)** were determined calorimetrically as measures of kidney functions.

C. Wound Healing Activity: The excision wound model was used **(KupeliAkkol *et al.*, 2009)**.Four groups of guinea pigs each of 6 were used. The dorsal skin of each animal was shaved, after 24 hours under ether anesthesia the shaved areas were sterilized (70% ethanol) and a predetermined dorsal area (15 mm x 20 mm and 2 mm depth) was inflicted. Local infection with *Staphylococcus aureus* was introduced into wounds using 0.5 mL of 10^8 bacteria mL^{-1} inoculums. The wounds were left undressed to the open environment and the animals were kept individually in separate cages. Wounds of the control animals were treated with the vehicle (1% v/v Tween 80). The other groups were treated with *C. elegans* (5 mg mL^{-1}), adenosine (1 mg mL^{-1}) and **latamoxef** (moxalactam)®(30 µg mL^{-1}). Treatments were applied topically on the wound surface once a day for 18 days.

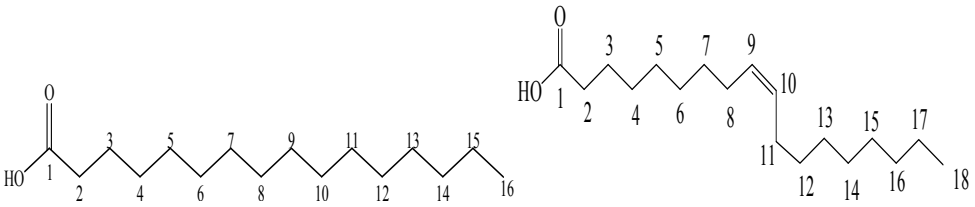
Assessment of wound healing: Under anesthesia, wound areas were measured on days 0, 4, 7, 10, 14, and 18. Wound contraction (%) was determined using the formula of **Lodhi*et al.*, (2006)**: Wound contraction (%) = [(WD0 - WDt) / WD0] 100

Where: WD0 = The wound diameter on day zero.WDt = The wound diameter on day t.

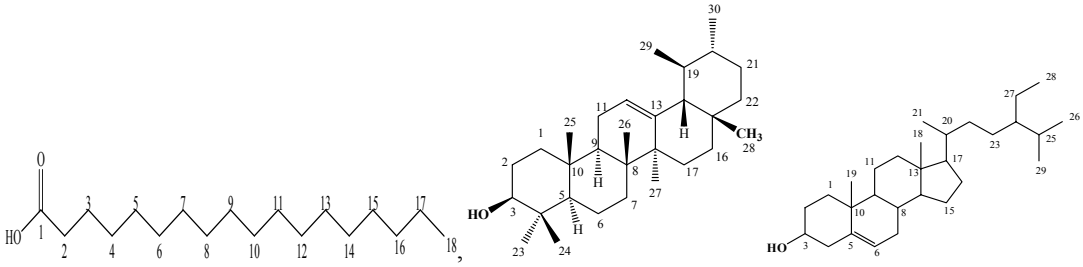
Statistical Analysis: All the values were expressed as mean ± S.E. Statistical analysis was done by using SPSS 10. Statistical significance of differences between two means was assessed by unpaired Student's 't' test. Differences at $p<0.05$, 0.01, and 0.001were considered statistically significant.

3. Results:

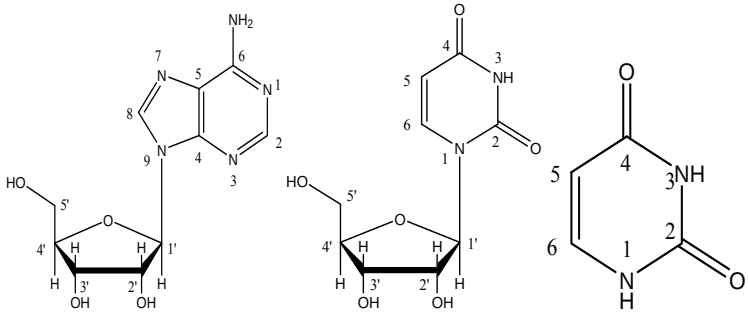
1) Isolated compounds: Three fatty acids were isolated and identified as Palmitic acid **1**, Oleic acid **2** and Stearic acid **3** in addition to other Seven compounds; α-amyrin **4**.β–sitosterol **5**, 2- (6-amino-9H-purin-9-yl) - 5- (hydroxymethyl) oxolane- 3, 4-diol(adenosine) **6**, Uridine (pyrimidine 2,4-dione,1-ribose) **7**,Pyrimidine-2, 4-dione (Uracil) **8**, 3,4,5-trihydroxy-benzoic acid (Gallic acid) **9**, and 3-(methoxycarbonyl) but-3-enoic acid **10** were isolated from the extract of *C. elegans* (figure 1) and identified using ^{1}HNMR, ^{13}CNMR, Heteronuclear multiple bond connectivity (HMBC),Heteronuclear multiple quantum Correlation (HMQC) and EI-MS.



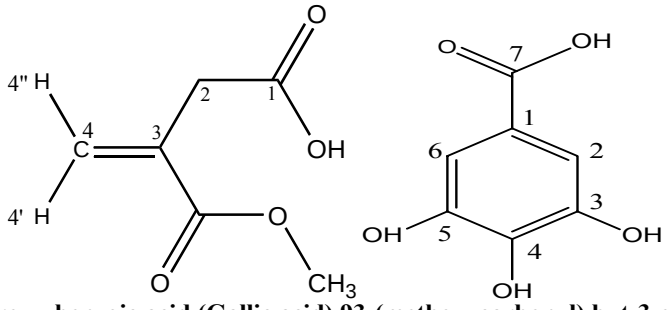
Palmitic acid 1 Oleic acid 2



Stearic acid 3 α-amyrin 4β–sitosterol 5



Adenosine6Uridine 7 Uracil,8



3,4,5-trihydroxy-benzoic acid (Gallic acid) 93-(methoxycarbonyl) but-3-enoic acid 10

Figure 1: The isolated compounds from *Cunninghamellaelegans*

2) Antimicrobial Activity

The obtained results revealed that the extract of the investigated fungus showed no activity against the tested unicellular fungi. However, there was a significance activity against Gram negative and/or Gram positive bacteria.

It also showed good activity against all the Gram-positive test organisms. However, it showed no activity against *Pseudomonas aeruginosa* and *Candida albicans* (Table 1).

The intra-cellular metabolites of *C. elegans* showed the highest activity against *Staphylococcus aureus* with zone of inhibition of (30.5±0.01) followed by the extra-cellular metabolites (29.3±0.03) against the same test organism and the extra-cellular metabolites (23.7±0.03) against the *bacillus subtilis*.

The antimicrobial activities of successive extracts of *C. elegans* were determined (Table 2). The highest activity was obtained by ethyl acetate extract against *Staph. Aureus* with zone of inhibition of (29.2±0.08), followed by ether extract (26.2±0.08). On the other hand, lowest activity was obtained by butanol extract (2.4±0.1), followed by ethyl acetate extract (3.4±0.5) against *Streptococcus pyogenes*.

The antimicrobial activity of compounds isolated from *C. elegans* were determined (Table 3). Only the test organism *Staph.aureus* was affected by the isolated compounds. However, the highest effect was obtained by compound **4** (adenosine) with zone of inhibition of (30.0±0.01), followed by compound **3** (Stearic acid) (15.0±0.5), and compound **1**(Palmitic acid) (13.0±0.1) (Table 3).

The minimum inhibitory concentration (MIC) of total alcohol, successive extracts and isolated compounds of *C. ellaelegans* against *Staph. aureus* was determined (Table 4).

The activity of *C. elegans* extract was (250 µg/ml). The best activity was obtained by the isolated compound adenosine (20 µg/ml), followed by ethyl acetate extract (50 µg/ml), and ether extract (100 µg/ml).

Table 1. Antimicrobial activity of *Cunninghamellaelegans* extract.

Test organisms	***Cunninghamellaelegans***		**Standard Antibiotics(1 mg/ ml)**			
	Intra	Extra				
Staphylococcus aureus	30.5±0.01	29.3±0.03	**Penicillin G**	29.5±0.8	**Streptomycin**	25.0±0.2
Bacillus subtilis	22.2±0.02	23.7±0.03		32.5±0.5		29.0±0.4
Streptococcus pyogenes	14.2±0.03	15.3±0.05		31.4±0.1		24.0±0.4
Pseudomonas aeurginosa	00.0	00.0		28.3±0.1		24.0±0.1
Escherichia coli	14.2±0.01	14.5±0.03		33.5±0.8		25.0±0.3
Salmonella typhimuim	09.2±0.01	11.5±0.03		34.3±0.4		28.0±0.3
Aspergillusfumigatus	13.9±0.03	14.3±0.03	**Itracon azole**	28.0±0.5	**Clotrim azole**	26.0±0.1
Penicilliumexpansum	15.2±0.04	13.4±0.03		27.0±0.1		23.0±0.3
Candida albicans	00.0	00.0		26.0±0.2		18.0±0.1

Table 2 Antimicrobial activity of successive extracts of *Cunninghamellaelegans*.

Test organisms	**Diameter of inhibition zone (mm)**								
	Petroleum ether	**Ether**	**$CHCl_3$**	**EAc**	**BuOH**	**Standard Antibiotics(1 mg/ ml)**			
Staphylococcus aureus	07.2±0.04	26.2±0.08	19.2±0.03	29.2±0.08	18.4±0.05	Penicillin G	29.5±0.8	Streptomycin	25.0±0.2
Bacillus subtilis	08.4±0.2	10.3±0.5	12.4±0.5	12.4±0.5	06.4±0.5		32.5±0.5		29.0±0.4
Streptococcus pyogenes	06.4±0.1	13.4±0.1	09.4±0.1	03.4±0.5	02.4±0.1		31.4±0.1		24.0±0.4
Pseudomonas aeurginosa	00.0	00.0	00.0	00.0	00.0		28.3±0.1		24.0±0.1
Escherichia coli	10.4±0.3	19.4±0.5	08.4±0.5	07.4±0.5	00.0		33.5±0.8		25.0±0.3
Salmonella typhimuim	09.4±0.1	20.4±0.1	09.4±0.1	11.4±0.5	00.0		34.3±0.4		28.0±0.3
Aspergillusfumigatus	09.2±0.08	10.2±0.08	13.2±0.08	06.4±0.5	05.3±0.5	Itraconazole	28.0±0.5	Clotrimazole	26.0±0.1
Penicilliumexpansum	10.4±0.5	11.4±0.5	10.4±0.2	04.4±0.5	00.0		27.0±0.1		23.0±0.3
Candida albicans	00.0	00.0	00.0	00.0	00.0		26.0±0.2		18.0±0.1

Table 3 Antimicrobial activity of the compounds isolated from *Cunninghamellaelegans*.

Test organisms / Compounds	Diameter of inhibition zone (mm)										
	1	2	3	4	5	7	8	Standard Antibiotics(1 mg/ ml)			
Staphylococcus aureus	13.0 ±0.1	11.0 ±0.3	15.0 ±0.5	30.0 ±0.1	11.0 ±0.1	05.0 ±0.5	07.0 ±0.5	**Penicillin G**	29.5±0 .8	**Streptomycin**	25.0 ±0.2
Bacillus subtilis	00.0	00.0	00.0	00.0	00.0	00.0	00.0		32.5 ±0.5		29.0 ±0.4
Streptococcus pyogenes	00.0	00.0	00.0	00.0	00.0	00.0	00.0		31.4 ±0.1		24.0 ±0.4
Pseudomonas aeurginosa	00.0	00.0	00.0	00.0	00.0	00.0	00.0		28.3 ±0.1		24.0 ±0.1
Escherichia coli	00.0	00.0	00.0	00.0	00.0	00.0	00.0		33.5 ±0.8		25.0 ±0.3
Salmonella typhimuim	00.0	00.0	00.0	00.0	00.0	00.0	00.0		34.3 ±0.4		28.0 ±0.3
Aspergillusfumigatus	00.0	00.0	00.0	00.0	00.0	00.0	00.0	**Itraconazole**	28.0 ±0.5	**Clotrimazole**	26.0 ±0.1
Penicilliumexpansum	00.0	00.0	00.0	00.0	00.0	00.0	00.0		27.0 ±0.1		23.0 ±0.3
Candida albicans	00.0	00.0	00.0	00.0	00.0	00.0	00.0		26.0 ±0.2		18.0 ±0.1

Palmitic acid **1**, Oleic acid **2** ,Stearic acid **3 ,** adenosine **4**, Uridine**5**,, Gallic acid **7**, and 3-(methoxycarbonyl) but-3-enoic acid **8**

Table 4 Minimum inhibitory concentration of extracts and isolated compounds of *Cunninghamellaelegans* against Staphylococcus aureus.

Extract	***C. elegans***									**Standard antibiotics**	
	Total extract	**Ether extract**	**Chloroform extract**	**Ethyl cetate extract**	**Butanol extract**	**Compound 4**	**Compound 5**	**Compound 7**	**Compound 8**	**Vancomycin**	**Gentamicin**
Minimum inhibitory concentration (µg/ml)	250	100	260	50	200	20	150	130	210	0.75	0.35

Compounds: adenosine **4**, Uridine**5**,,Gallic acid **7**, and 3-(methoxycarbonyl) but-3-enoic acid **8**

Table 5 Effect of prolonged oral administration of the ethanol extracts of *C. elegans*(200 mg kg^{-1}) for 35 days on the serum concentration of different liverand kidney markers in rats (n=6).

Groups/ Marker	**Control**	***C. elegans***
Total bilirubin (mg dl^{-1})	0.28±0.02	0.31±0.02
Total proteins (g dl^{-1})	6.44±0.28	6.51±0.30
Albumin (g dl^{-1})	4.13±0.20	4.35±0.22
Globulin (g dl^{-1})	2.31±0.16	2.16±0.15
AST (U L^{-1})	80.6±5.22	88.6±5.75
ALT (U L^{-1})	46.2±2.74	50.0±3.55
ALP (U L^{-1})	88.5±5.20	94.0±6.22
Uric acid (mg dl^{-1})	2.18±0.21	2.32± 0.20
Urea (mg dl^{-1})	45.4±2.54	48.8±3.90
Creatinine (mg dl^{-1})	0.66±0.05	0.61±0.05

Table 6 Effect of topical application of total alcohol extract of *C. elegans* (5 mg mL^{-1}), adenosine (1 mg mL^{-1}) and latamoxef (30 µg mL^{-1}) on the wound area of *Staphylococcus aureus*infected wounds in guinea pig. (n=6).

Treatment	Wound area (mm^2) on day					
	0	4	7	10	14	18
Control	300±0.0	264.6±9.55	205.2±9.45	152.5±6.38	95.3±3.47	32.4±1.66
Total alcohol extract	300±0.0	235.8±9.14*	165.6±6.88**	83.4±3.16***	22.4±1.15***	0.0±0.0
Adenosine	300±0.0	241.2±4.23*	168.1±2.18**	85.6±4.13***	23.3±1.11***	0.0±0.0
Latamoxef	300±0.0	219.4±9.37**	146.4±5.60***	42.6±1.85***	0.0±0.0	-

Statistically significant at: *P<0.05;,**P<0.01; *** P<0.001.

3) Pharmacological activities:

a- **Acute Toxicity Study:** Mice of all groups survived for 24 hours with no signs of toxicity.

b- **Sub-chronic toxicity:** In the present study rats of the treated groups did not show any significant effect on the activity of AST, ALT, ALP, total bilirubin, total protein, albumin, globulin, uric acid, urea and creatinine in their sera as compared to control (Table 5).

Wound Healing Activity: The results of present study revealed that the topical application of *C. elegans* methanol extract (5 mg mL^{-1}) and isolated compound adenosine (1 mg mL^{-1}) on the experimentally excised wound surface accelerated the wound healing process (Table 6). A remarkable increase in the percentage of wound contraction was observed in adenosine treated guinea pigs as compared with the control. Topical application of adenosine showed a 76.50% contraction on Day 14, which was close to the contraction value of the reference drug; **latamoxef** (100%). Complete wound contraction took place in adenosine treated animals; 4 days after those treated with the reference drug. A drug to be used for effective wound healing should be able to clear the wound by the 19thday after infliction (Esimone*et al*., 2005). No mortality was noticed amongst the animals in all the three groups.

4. Discussion

The Fungi have been the major source of the most important antibiotics in human history for decades. Although terrestrial fungi become a focal point of interest for natural product chemists since they are rich in a wide variety of secondary metabolites such as tannins terpenoids, alkaloids, flavonoids, etc., which have been found in vitro to have antimicrobial properties (**Zain 2011, Samuel et al. 2011, Siddhardha et al. 2009**). The results obtained from the current study revealed that the total alcoholic extract of *Cunninghamellaelegans*showed promising antibacterial and antifungal activities. Previous study revealed that the bioactive metabolites from solvents and crude extract of fungal species such as *Aspergillusflavus*, *Geotrichumcandidum*, *Penicilliumleuteum*, *P. granulatum* and *Acremonium* species showed antibacterial activity against five human pathogenic bacteria (**Samuel et al 2011**). Inthis study, the biological evaluation of eight compounds isolated from *Cunninghamellaelegans* towards different types of bacterial speciesshowed good activity against *Staphylococcus aureus* similar result are obtained by **Awaad et al. (2012).** It means that this fungus produce antibacterial compounds, however, the secondary metabolites does not occur randomly but is correlated with one or more ecological factor. Nevertheless, a growing number of metabolites from fungal strains has been reported in the last years (**Zain et al., 2014**).

The tested extracts considered to be highly safe since substances possessing LD_{50} higher than 50 mg kg^{-1} are non-toxic (**Buck *et al*., 1976**).

The serum transaminase level is the most widely used as a measure of hepatocyte injury, due to its ease of measurement and high degree of sensitivity (**Johnston, 1999**). It is useful for the detection of early damage of hepatic tissue. Therefore, the evaluated extract is not hepatotoxic.

Urea and creatinine are the most sensitive biochemical markers employed in the diagnosis of renal damage. Therefore marked increase in serum urea and creatinine are indications of functional damage to the kidney (**Panda, 1999and El-Meligyet al., 2014).**

Wounds occur when the continuity of the skin or mucous membrane is broken (**Ross and Wilson, 1970**). Initially all guinea pigs had wounds of similar areas (300 mm^2) which were accomplished by using stencil. Wound healing involves regeneration of specialized cells by proliferation of surviving cells and connective tissue response characterized by the formation of granulation tissue (Whaley and Burt, 1996).

Conclusively, adenosine is safe, showed antimicrobial activity against some microbes and exhibited good wound healing effect comparable to those of **latamoxef**, a standard antibiotic used in wound healing.

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Conflict of interest

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the paper.

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