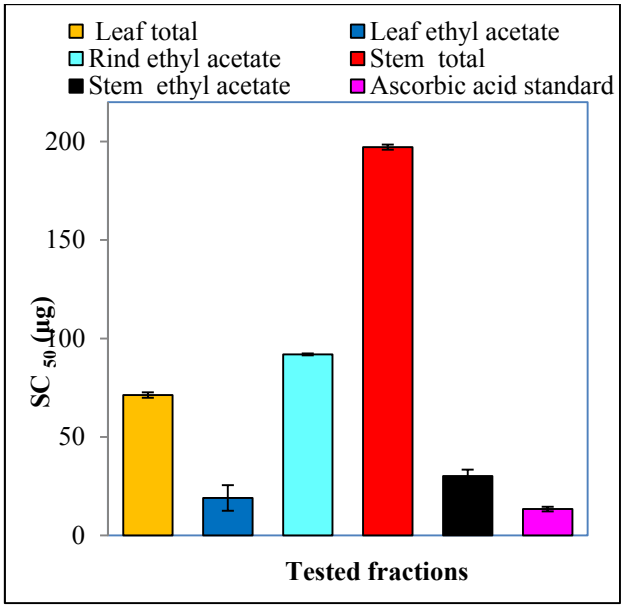

Figure 3: SC_{50} of pink lemon fractions and ascorbic acid.

3.6. Antimicrobial activity

Results of antibacterial and antifungal activity of total alcoholic extract and different fractions against different microorganisms by well diffusion technique were expressed as diameter of inhibition zone and percentage of activity (Table 3). Juice and stem *n*-hexane, and rind dichloromethane fractions didn't inhibit the growth of the all tested bacteria and fungi. These results also indicated that ethyl acetate fraction of juice (JE) showed MIC values 0.49 µg/ml that is equal to MIC of ampicillin against both *E. faecalis* and *B. subtilis*. The potential use of ethyl acetate fractions of leaf (LE) and rind (RE) in management of bacterial diseases caused by *K. pneumonia* as it showed strong antibacterial activities with MIC = 1.95 µg/ml which exceeded the activity of gentamicin with MIC = 3.9 µg/ml (Figure 4). Only the total extract of leaf could exhibit antifungal activity against *C. albicans,* reached to 92.69% activity of amphotericin B with MIC = 0.98 µg/ml. The best antifungal activity was observed for ethyl acetate of juice and rind against *S. cerevisiae* (MIC = 0.98 µg/ml compared to amphotericin (MIC = 0.24 µg/ml).

In a previous report, methanolic extract of *C. limetta* (sweet lime) peel was more effective compared to other extracts as ethyl acetate, chloroform and water in their anti-microbial activity against the pathogenic *E. coli, Pseudomonas sp., Klebsiella sp*. and methicillin resistant *S. aureus* due to the presence of flavones and phenolic contents [93]. Moreover**,** *C. medica* L. peel extract and the juice of the ripen and unripe fruit of *C .limon* possessed significant antimicrobial activity against *S. aureus*, *Klebsiella sp., E. coli, P. aeruginosa* and *C. albicans* [94, 95].

Table 3: Results of the antimicrobial screening of the different total alcoholic extract and fractions of pink lemon

Tested material	Inhibition zone diameter (mm ±S.D) (% of inhibition)								
	G-ve			G+ve			Fungi		
	E. cloacae	*K. pneumoniae*	*E. coli*	*S. aureus*	*E. faecalis*	*B. subtilis*	*A. fumigatus*	*C. albicans*	*S. cerevisiae*
Ampicillin	-	-	-	28.9 ± 0.14 (100)	25.3 ± 0.58 (100)	26.4 ± 0.34 (100)	-	-	-
Gentamicin	23.8 ± 0.63 (100)	20.2 ± 0.12 (100)	27.3 ± 0.44 (100)	-	-	-	-	-	-
Amphotricin B	-	-	-	-	-	-	23.7 ± 0.10 (100)	21.9 ± 0.12 (100)	27.8 ± 0.58 (100)
JC	17.8± 0.63 (74.79)	16.4 ± 0.37 (81.19)	18.5 ± 0.58 (67.77)	16.9 ± 0.58 (58.48)	18.3 ± 0.44 (72.33)	20.4 ± 0.44 (77.27)	16.2 ± 0.58 (68.35)	NA	18.4 ± 0.58 (66.19)
JE	21.7 ± 0.36 (91.18)	18.9 ± 0.25 (93.56)	20.4 ± 0.63 (74.73)	21.9 ± 0.44 (75.78)	23.7 ± 0.37 (93.68)	24.2 ± 0.25 (91.67)	19.8 ± 0.25 (83.54)	NA	21.7 ± 0.25 (78.06)
JT	22.4 ± 0.25 (94.12)	20.3 ± 0.37 (100.50)	19.8 ± 0.63 (72.53)	21.4 ± 0.58 (74.05)	22.2 ± 0.44 (87.75)	23.8 ± 0.58 (90.15)	20.6 ± 0.58 (86.92)	NA	21.3 ± 0.63 (76.62)
SC	16.2 ± 0.44 (68.07)	15.8 ± 0.12 (78.22)	18.2 ± 0.44 (66.67)	16.2 ± 0.37 (56.06)	17.4 ± 0.44 (68.77)	18.3 ± 0.37 (69.32)	18.2 ± 0.25 (76.79)	NA	18.9 ± 1.2 (67.99)
SE	16.8 ± 0.58 (70.59)	14.2 ± 0.25 (70.30)	17.6 ± 0.63 (64.47)	17.4 ± 0.44 (60.21)	19.3 ± 0.37 (76.28)	20.1 ± 0.25 (76.14)	16.7 ± 0.25 (70.46)	NA	20.3 ± 0.25 (73.02)
ST	21.3 ± 0.25 (89.50)	18.7 ± 0.44 (92.57)	20.3 ± 0.63 (74.36)	18.2 ± 0.44 (62.98)	19.8 ± 0.58 (78.26)	21.2 ± 0.37 (80.30)	17.3 ± 0.58 (73.00)	NA	19.2 ± 0.58 (69.06)
RH	18.3 ± 0.58 (76.89)	16.8 ± .37 (83.17)	17.3 ± 0.58 (63.37)	16.9 ± 0.58 (58.48)	16.5 ± 0.44 (65.22)	18.4 ± 0.44 (69.7)	14.2 ± 0.58 (59.92)	NA	14.2 ± 0.58 (51.08)
RE	22.4 ± 0.44 (94.12)	19.9 ± 0.25 (98.51)	21.3 ± 0.44 (78.02)	19.3 ± 0.58 (66.78)	21.4 ± 0.63 (84.58)	23.2 ± 0.58 (87.88)	21.3 ± 0.44 (89.87)	NA	22.4 ± 0.44 (80.58)
RT	19.7 ± 0.48 (82.77)	16.5 ± 0.37 (81.68)	17.6 ± 0.25 (64.47)	17.4 ± 0.25 (60.21)	17.9 ± 0.58 (70.75)	19.2 ± 0.44 (72.73)	19.1 ± 0.63 (80.59)	NA	21.2 ± 0.58 (76.26)
LH	NA	NA	NA	13.7 ± 0.58 (47.40)	14.1 ± 0.58 (55.73)	16.3 ± 0.32 (61.74)	NA	NA	NA
LC	20.1 ± 0.63 (84.45)	17.8 ± 0.63 (88.12)	19.2 ± 0.72 (70.33)	19.3 ± 0.44 (66.78)	19.8 ± 0.44 (78.26)	20.4 ± 0.36 (77.27)	20.4 ± 0.58 (86.08)	NA	21.2 ± 0.58 (76.26)
LE	22.4 ± 0.58 (94.12)	20.8 ± 0.19 (102.97)	21.3 ± 0.58 (78.02)	20.9 ± 0.44 (72.32)	18.6 ± 0.58 (73.52)	21.9 ± 0.36 (82.95)	20.4 ± 0.58 (86.08)	NA	20.4 ± 0.58 (73.38)
LT	17.1 ± 0.63 (71.85)	18.6 ± 0.24 (92.08)	20.5 ± 0.58 (75.09)	20.3 ± 0.58 (70.24)	20.9 ± 0.58 (82.61)	21.2 ± 0.32 (80.30)	17.2 ± 0.32 (72.57)	20.3 ± 0.58 (92.69)	21.4 ± 0.32 (76.98)

JC: Juice dichloromethane fraction, JE: Juice ethyl acetate fraction, JT: Juice total extract, SC: Stem dichloromethane fraction, SE: Stem ethyl acetate fraction, ST: Stem total extract, RH : Rind *n*-hexane fraction, RE: Rind ethyl acetate fraction, RT: Rind total fraction, LH : Leaf *n*-hexane fraction, LC: Leaf dichloromethane fraction, LE: Leaf ethyl acetate fraction , LT: Leaf total fraction.NA: No activity

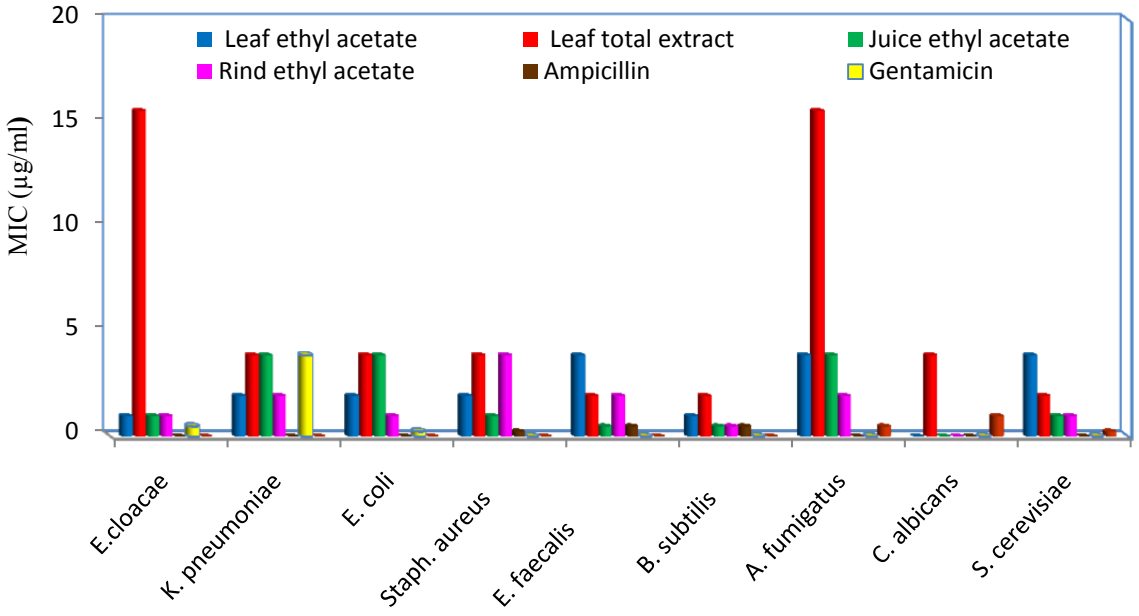

Figure 4: Minimum inhibitory concentration (MIC) of pink lemon fractions, antibiotics and antifungal.

Fruit juice of *C. limon* and *C. aurantium* showed high antimicrobial activity against *E. coli*. Their activity is probably due to the presence of flavonoids and steroids that are able to complex with extracellular and soluble proteins and to complex with bacterial cell wall [96]. Additionally, methanolic extract of *C. aurantifolia* leaves showed high potency against *E. coli* and *S. aureus* using erythromycin as positive control

The antibacterial activity may be attributed to the presence of flavonoids, tannins and steroidal alkaloids [97]. Moreover, ethyl acetate extract of lemon seeds (*C. limon*) showed its maximum activity against *E. coli* while hot water extract of lemon peel was found to be maximum against *S. aureus* [98]

In a previous report, chrysoeriol showed a strong inhibitory effect on *K. pneumonia* and *B. subtilis* and to less extent against *E. cleacae*, *E. coli*, *S. aureus* and *C. albicans* [99]. LC-MS analysis indicated the presence of apigenin in LE and vitexin in stem ethyl acetate (SE) and RE. Basile et al. [100] confirmed that apigenin and vitexin exhibited antimicrobial activities against *E. coli, K. pneumoniae*, and *E cloacae*, which is in agreement with our results.

β-Sitosterol and oleic acid extracted from flavedo and albedo of *C. grandis* Osbeck showed higher antimicrobial activity than limonin against *E. coli*, *B. subtilis* and *S. aureus* which may be attributed to the synergistic effects of *β*- sitosterol and oleic acid [101].

C. grandis carotenoids extract was suggested as a natural alternative for chemicals in food preservation as *β*- carotene could lead to the accumulation of lysozyme, an antibacterial immune enzyme that digests bacterial cell walls [102].

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CONCLUSION

Thirteen compounds were isolated and characterized for the first time in variegated pink-fleshed eureka lemon cultivated in Egypt. Organic acids and their glycosides, flavonoids and their HMG derivatives, coumarins and limonoids were identified in different organs using HPLC-PDA-ESI-MS/MS analysis. Ethyl acetate fraction and total alcoholic extract of the stem showed the highest concentration of phenolics and flavonoids, respectively while leaf ethyl acetate fraction showed the highest *in vitro* antioxidant activity compared with other tested fractions. Juice and rind ethyl acetate fractions exhibited the best antimicrobial activities. This study recommended the consumption of variegated pink lemon and its use as food additive of natural origin or pharmaceutical supplement products.

CONFLICT OF INTEREST

The authors declared no conflict of interest

ACKNOWLEDGMENT

The authors are grateful to Prof. M. Wink and Dr. M Sobeh, institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany for carrying out LC-MS analysis and Ass. Prof. M. E. Mohamed, Department of Pharmaceutical Sciences, School of Clinical Pharmacy, University of King Faisal, Ahsaa, Kingdom of Saudi Arabia for doing some of NMR analysis.

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