Table 1: Effect of active fraction of *A. pavonina* on the viability of the bacterial cells in the culture medium as estimated by the standard plate count method after 24 h incubation. Values are mean±SD, *n*=4

Concentration of AEF (mg/ml)	10^8 CFU/ml	
	C. violaceum 12472	*P. aeruginosa* PAO1
Control	2.48±0.02	2.33±0.04
Solvent control	2.28±0.03	2.37±0.05
0.25	2.15±0.02	2.15±0.03
0.5	2.28±0.03	2.18±0.02
1.0	2.19±0.01	2.23±0.01
2.0	2.07±0.02	2.09±0.01

AEF=Active fraction; SD=Standard deviation

Table 2: Inhibition of swarming motility in *Pseudomonas aeruginosa* PAO1 by different concentrations of active fraction of *A. pavonina*. Values are mean±SD. *n*=4. Same letters in the columns are not significantly different (*P*<0.001)

Concentration of AEF (mg/ml)	Swarming diameter (mm)
Control	65.75±0.95
0.10	09.25±1.50[a]
0.25	07.50±1.29[b]
0.50	05.00±1.08[c]
1.00	05.00±1.05[c]

AEF=Active fraction; SD=Standard deviation

inhibition of elastolytic and proteolytic activities in *P. aeruginosa* PAO1. Since biofilm formation is partially controlled by QS mechanisms, the effect of AEF on biofilm formation in *P. aeruginosa* PAO1 was assessed after 18 h of growth. At 1.0 mg/ml concentration of AEF, the biofilm formation was decreased by 81% [Figure 3a].

Phytochemical screening of anti-QS compounds separated by TLC

The AEF fraction was spotted onto a silica gel TLC plate and eluted with chloroform: Methanol, after which the TLC plate was cast into agar containing *C. violaceum* CV026 biosensor strain. After incubation, a band showing anti-QS zone was observed with R_f value 0.63 [Figure 1b].

DISCUSSION

QS plays an important role in the regulation of cell physiology in many Gram-negative bacteria. QS system consisted of inducer and regulator proteins of las and rhl components, which work interdependently in a hierarchal manner to regulate the expression of various genes, including virulence ones in *P. aeruginosa*.[17] In the present study, *A. pavonina* inhibited of both las- and rhl-mediated

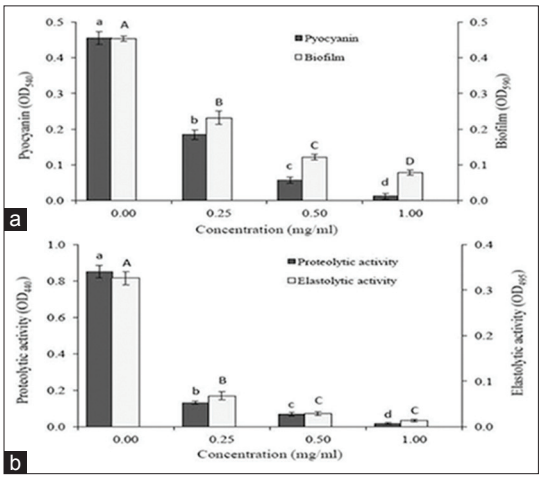

Figure 3: The effect of AEF on QS-regulated virulence factor production in *P. aeruginosa* PAO1. (a) Pyocyanin production and biofilm formation (b) Proteolytic activity and elastolytic activities. Values are expressed as mean ± SD, *n* = 4. Same letters in the columns (bar) are not significantly different (*P* < 0.001)

phenotypes in *P. aeruginosa* PAO1. Preliminary screening of anti-QS activity was carried out using *C. violaceum* CV026 biosensor strain. In *C. violaceum* CV026 plate assay, formation of halo zone indicates that *A. pavonina* is either inhibiting the C_6-AHL competitively from binding to its transcriptional regulator, CviR; degrading the C_6-AHL enzymatically, or removing the C_6-AHL via active transport.[18,19] Inhibition of violacein production was quantified and from the results obtained in this study, it was proven that *A. pavonina* reduced violacein production significantly. In agreement to this finding, other plant extracts of *Conocarpus erectus*, *Quercus virginiana* and other higher plants have demonstrated the anti-QS activity against biosensor strain *C. violaceum* CV026.[20]

In *P. aeruginosa*, the production of pyocyanin is under the control of rhlI-rhlR QS system. Pyocyanin is highly permeable to the biological membrane and causes extensive cellular damage in the lungs of cystic fibrosis patients. Secretion of elastase and protease enzymes is also an important aspect of pathogenicity, which helps in combating adverse conditions and tissue colonization inside the host.[21] *P. aeruginosa* exhibits swarming motility, which helps in initial attachment and later in relocation of biofilm from one site to another.[22] In the present study, significant reduction in the pyocyanin production, proteolytic and elastolytic activities, swarming motility, and biofilm formation in *P. aeruginosa* PAO1were observed in the presence of AEF of *A. pavonina*.

The anti-QS compound present in the active fraction of *A. pavonina* was separated by TLC. Phytochemical analysis of *A. pavonina* plant is still in its primitive stages; exploration of its activity may invite further studies on the phytochemicals

present. Tannin-rich fraction from *Terminalia catappa* showed inhibition of QS-mediated virulence factor production in *C. violaceum* and *P. aeruginosa* PAO1.[23] Flavonoids, catechin and naringin also been proved to inhibit QS in Gram-negative pathogens.[24] Some known mechanisms of QS inhibition include competitive binding of signal-like molecules to cognate receptors, as in the case of furanones, enzymatic degradation of QS signals, as in the case of AHL acylases. Further studies are needed to demonstrate the exact mechanism of QS inhibition by *A. pavonina* phytocompounds. It is interesting to study the important mechanism involved by the plants used in traditional phytomedicine and revealing the possible mechanism/mode of action shall aid in the invention of lead molecules for the antimicrobial drugs.

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