Supplementary information

Promising protective treatment potential of endophytic bacterium *Rhizobium aegyptiacum* for ulcerative colitis in rats

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Materials and methods

1 Liquid chromatography-mass spectrometry

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis of endophytic bacteria *R. aegyptiacum* extract was performed in the Proteomics and Metabolomics Unit, Children's Cancer Hospital (57357), adopting methods previously reported (Attallah et al., 2022; Elmongy et al., 2022). Positive and negative electrospray ionization approaches were utilized to detect the various phytoconstituents of the endophytic bacteria *R. aegyptiacum* extract.

2 Induction of ulcerative colitis (UC)

Acetic acid (AA) was used to induce acute colitis in rats. Rats were fasted for 16 h with free access to water and were administered 5% AA intra rectal injection of a single dose with a 6G nelaton catheter, under mild anesthesia (ketamine hydrochloride (50 mg/kg) and xylazine (5 mg/kg) mixture). A catheter was inserted 6 cm into the anus, and one milliliter of AA, after which rats were maintained in the Trendelenburg position for 15 min. Further, to neutralize the severity of the AA solution, each rat rectum was washed with saline (0.5 mL)

3 Scanning electron microscopy (SEM)

Colonic tissues were fixed for two hours at room temperature in 2.5% buffered glutaraldehyde in 0.1 mol/L PBS pH 7.4 and then rinsed three times with PBS for ten minutes each. Post fixation in 1% osmic acid for 30 min came next, and then ethyl alcohol infiltrated with acetone was used to dehydrate the samples. The samples were dried in a SPI Supplies® critical point drying machine using liquid CO_2, mounted on aluminum stubs, coated with gold in a SPI-Module TM Vac/Sputter. photography using JEOL, JSM-5200 LV scanning electron microscope, Japan, electron microscope unit, Tanta University (Abbate et al., 1993).

References

Abbate, F., R. Laura, U. Muglia, G. Vita and P. Bronzetti (1993). "Differentiation of ependymal surface of lateral ventricles in fetus and newborn rabbits: Observations by SEM." Anatomia, Histologia, Embryologia **22**(4): 348-354.

Attallah, N. G., S. A. El-Sherbeni, A. H. El-Kadem, E. Elekhnawy, T. A. El-Masry, E. I. Elmongy, N. Altwaijry and W. A. Negm (2022). "Elucidation of the Metabolite Profile of Yucca gigantea and Assessment of its Cytotoxic, Antimicrobial, and Anti-Inflammatory Activities." Molecules **27**(4): 1329.

Elmongy, E. I., W. A. Negm, E. Elekhnawy, T. A. El-Masry, N. G. Attallah, N. Altwaijry, G. E.-S. Batiha and S. A. El-Sherbeni (2022). "Antidiarrheal and antibacterial activities of Monterey cypress phytochemicals: in vivo and in vitro approach." Molecules **27**(2): 346.

Table S1 Primers utilized in the current study

Gene	Primer sequence 5' to 3'
β-actin	AGGCGTCCTTCCTTATATGCTA
	GGCTGTATTCCCCTCCATCG
HO-1	AGGTACACATCCAAGCCGAGA
	ATCACCAGCTTAAAGCCTTCT
TNF-α	CTGAGGTCAACCTGCCCAAGT
	GAGAACGGATGAACACGCCAGT

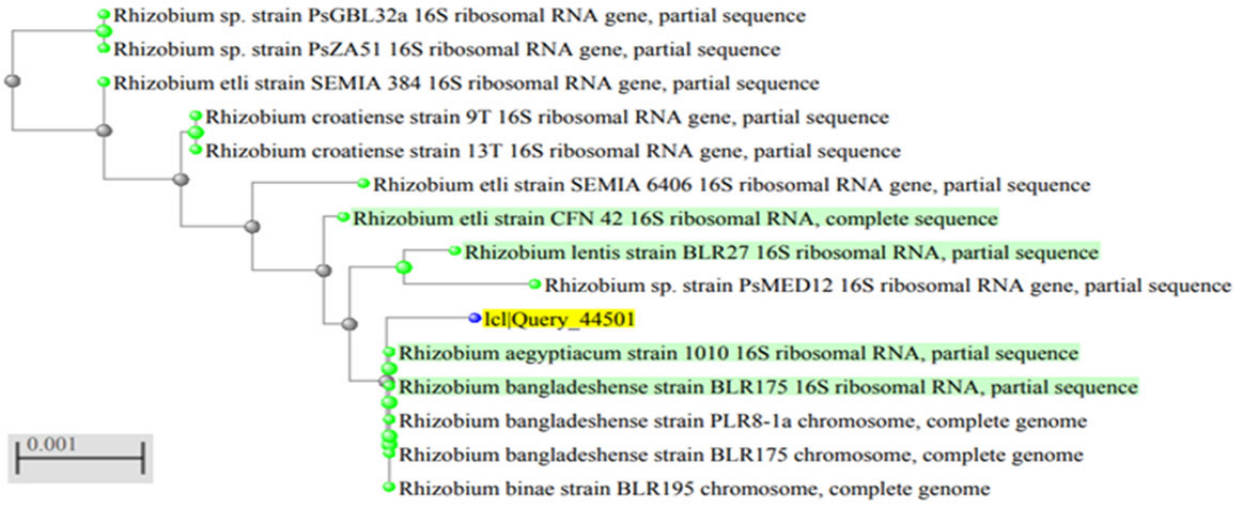

Fig. S1 Phylogenetic tree of the isolated bacteria based on the 16S rRNA for sequencing. The yellow color indicates the identified bacterial isolate (*R. aegyptiacum*).

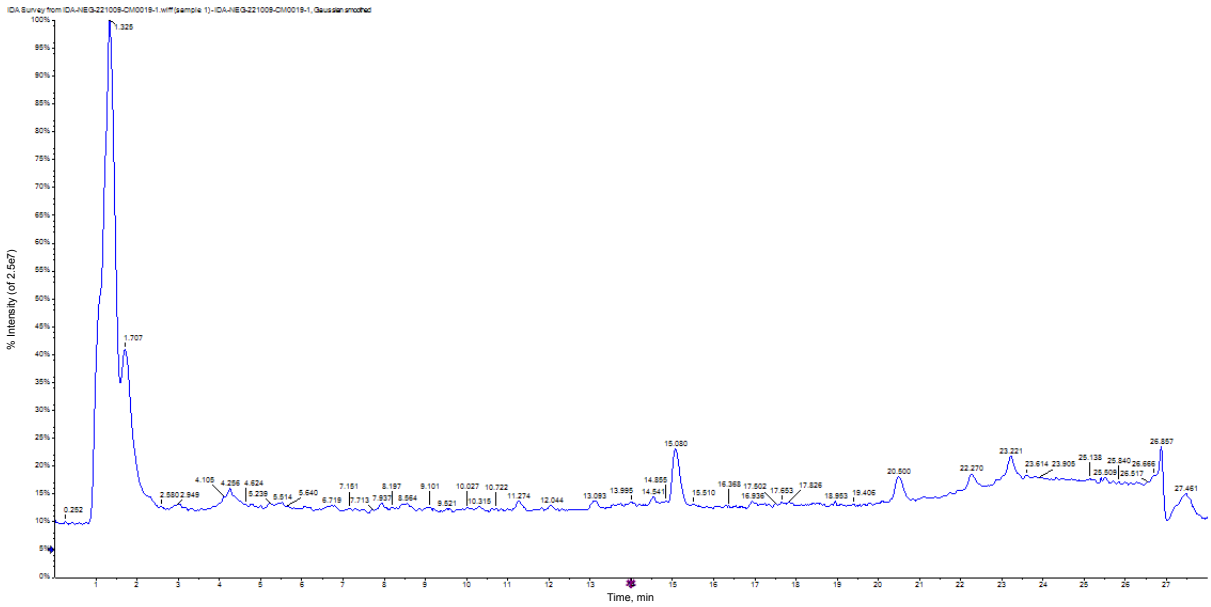

Fig. S2 Total ion chromatogram of detected metabolites of *R. aegyptiacum* extract in negative ion mode.

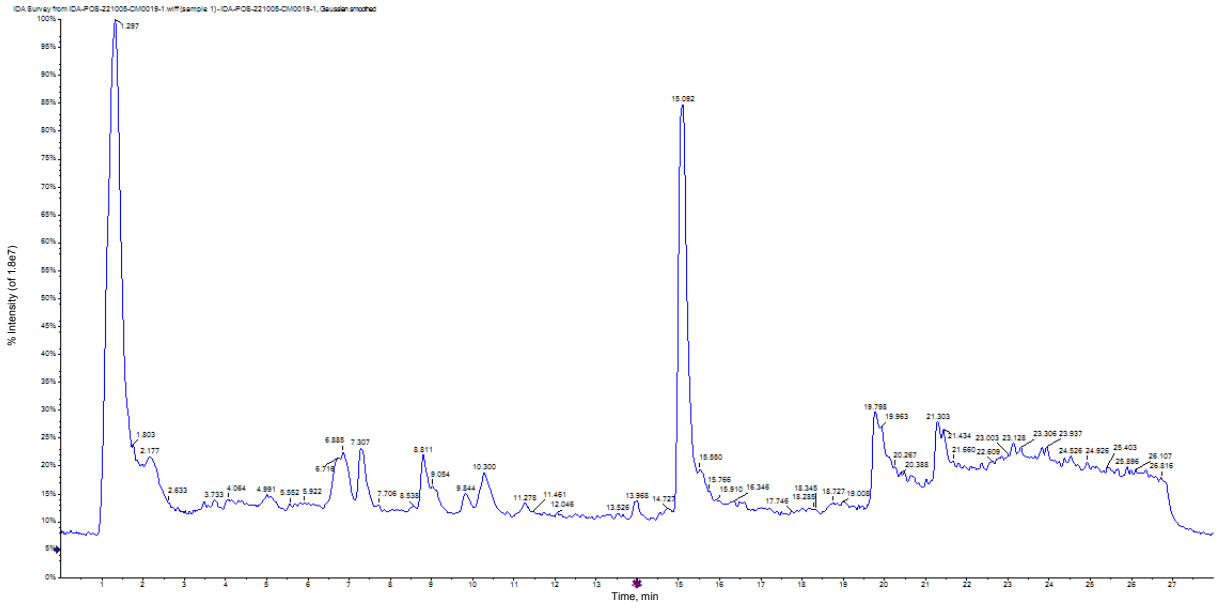

Fig. S3 Total ion chromatogram of detected metabolites of *R. aegyptiacum* extract in positive ion mode.