The yield of oil isolated by hydrodistillation from aerial parts of Thymus fontanesii Boiss. et Reut. (Lamiaceae) growing wild in Djelfa (Algeria) was 0.9%. Analysis of the oil by gas chromatography (GC) and gas chromatography/mass spectrometry (GC/MS) revealed the identification of 47 components accounting for 98.5% of the total oil. The oil was found to be rich in monoterpenes (91.5%) with major constituents being thymol (29.3%), γ-terpinene (21.7%), p-cymene (15.9%), and thymol methyl ether (11.4%), while a smaller amount of linalool (4.8%) and β-caryophyllene (2.9%) were detected. According to the antimicrobial study using the disk diffusion method and the agar dilution assay-minimal inhibitory concentration (MIC), the oil showed strong in vitro growth inhibition activity against Gram-negative bacteria and antifungal activity. The oil exhibited the maximum antifungal activity against Mucor ramaniamus (MIC = 0.2 µL/mL)