

New γ -pyrone derivative from *Helichrysum sanguineum* (L.) and its Anti-*Helicobacter pylori* activity

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Short Report

Keywords: Peptic ulcer, Antibacterial, Natural products, Medicinal plants

Posted Date: August 4th, 2025

DOI: <https://doi.org/10.21203/rs.3.rs-7059138/v1>

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Version of Record: A version of this preprint was published at Revista Brasileira de Farmacognosia on March 18th, 2026. See the published version at <https://doi.org/10.1007/s43450-025-00725-w>.

Abstract

Helichrysum sanguineum is a medicinal plant traditionally used for treatment of several diseases.

Phytochemical investigation of its aerial parts led to isolation of four compounds, including one new γ -pyrone derivative named Kadipyrone (**1**) and identified as 2-(4-hydroxy-6-methylheptan-2-yl)-3-methyl-4*H*-pyran-4-one. Three known compounds were also isolated including luteolin (**2**), quercetin (**3**) and para hydroxy benzoic acid (**4**). Structures of these compounds were elucidated through extensive spectroscopic methods, including ESI-MS, HR-ESI-(qTOF)-MS, ^1H NMR, ^{13}C -NMR, APT-NMR experiments and two dimensional NMR techniques (HSQC and HMBC). Further, different fractions obtained from *Helichrysum sanguineum* and compound **1** were evaluated for their anti-bacterial activity against *Helicobacter pylori* through diffusion assay. The results indicated that compound **1** has a promising activity toward *H. pylori* with a minimum inhibitory concentration (MIC) of 32 $\mu\text{g/ml}$, suggesting the potential role of compound **1** in the treatment of *H. pylori* infection.

Introduction

Helicobacter pylori is one of the most pathogenic bacteria infecting humans, with approximately 50% of populations worldwide are being infected with this gram negative bacteria. Prevalence rates exceed 70% in developing countries (Hooi et al., 2017). *H. pylori* infection can lead to serious gastrointestinal complications, including peptic ulcers, duodenal ulcers, and gastric cancer (Reyes, 2023), as well as several extra-gastric disorders such as cardiovascular diseases, diabetes mellitus, anemia, neurologic disorders, metabolic disorders and liver diseases (Gravina et al., 2018). The ability of *H. pylori* to survive in various harsh microenvironments contributes to its significant resistance to multiple antimicrobial agents (de Souza Gonçalves et al., 2021). Several studies have demonstrated the efficacy of various natural products and phytochemicals in treating and eradicating of *H. pylori* infections (Deng et al., 2024). Within natural-derived products, γ -pyrone compounds are one of the most potent anti-*H. pylori* agents, and their activities have been investigated in previous studies (Taniguchi et al., 2000). Of note, some γ -pyrones have been previously isolated from *Helichrysum* genus (Werner et al., 2019). The genus *Helichrysum* is a large genus in the Asteraceae family and comprises approximately 600 species that are distributed worldwide. *Helichrysum sanguineum* (L.) Kostel, commonly known as Red Everlasting, is a perennial herbaceous species native to Mediterranean and Middle East regions, including Turkey, Lebanon, Palestine and Syria (Bahadırli et al., 2024). Traditional medicinal applications of *H. sanguineum* are well-documented across Mediterranean regions, including Turkey and Palestine, where aqueous decoction has been employed to treat asthma, tuberculosis, urogenital disorders, hepatobiliary conditions (jaundice), dermatological inflammation, diarrhea, renal calculi, and fever (Bahadırli et al., 2024). Although numerous studies have been conducted on *Helichrysum* species, several species including *H. sanguineum* remain poorly investigated. In this work, the chemical composition and antimicrobial activity of dichloromethane and ethyl acetate fractions of the aerial parts of *H. sanguineum* were investigated.

Material and Methods

About 2.5 kg of dried aerial parts of *H. sanguineum* were collected in April 2023 from a local Egyptian herbal market (originally imported from Syria). The plant material was authenticated by Prof. Dr. Abdo Marei, Professor of Plant Ecology, Department of Botany and Microbiology, Faculty of Science, Al-Azhar University. A voucher specimen (HS-180) was deposited in the Herbarium of the Department of Pharmacognosy and Medicinal Plants, Al-Azhar University, Cairo, Egypt. The plant material was grinded and extracted by maceration with 70% methanol (3×10L), followed by evaporation under reduced pressure to give a 253 g crude extract. The crude extract was then suspended in 500 ml distilled water and partitioned with *n*-Hexane (3L), dichloromethane (DCM, 3L), ethyl acetate (EtOAc, 3L) and *n*-butanol (*n*-BuOH, 6L) to give corresponding fractions of 0.25, 3, 15 and 42 g, respectively.

The antimicrobial activity of these fractions was tested against gram-positive bacterial strains including *Staphylococcus epidermidis* and *Bacillus subtilis*, gram negative bacterial strains including *Helicobacter pylori*, *Salmonella typhimurium*, and *Serratia marcescens*, as well as against fungi including one filamentous fungus, *Aspergillus fumigatus*, and one yeast species, *Candida tropicalis*. All microbial strains, except *H. pylori*, were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt. The antimicrobial activities were evaluated using a modified well diffusion method (Rasras et al., 2023). Briefly, 100 µl of the test bacteria/fungi were grown on 10 ml of fresh media until they reached a count of approximately 10^8 cells/ml for bacteria or 10^5 cells/ml for fungi. One hundred µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by well diffusion method. One hundred µl of each sample (at 10 mg/ml) was added to each well (6 mm diameter holes cut in the agar gel). The plates were incubated for 24–48 h at 37°C (for bacteria and yeast) and for 48 h at 28°C (for filamentous fungi). After incubation, the microorganism's growth was observed. The resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. Solvent control (DMSO) was included in every experiment as negative controls. Positive controls were also performed using gentamycin as standard antibacterial drugs and ketoconazole as standard antifungal drug. The *H. pylori* standard strain American Type Culture Collection (ATCC 26695) was obtained from the Department of Microbiology and Immunology, Faculty of Pharmacy (Men), Al-Azhar University. *H. pylori* was cultured on selective media (Columbia agar with 5% sheep blood and antibiotics) under microaerophilic conditions (5–10% O₂, 5–10% CO₂, 80–90% N₂) for 3–5 days (Ma et al., 2024). The anti-bacterial activity of the *H. sanguineum* fractions and the compound 1 against *H. pylori* was determined at the laboratory of microbiology and immunology Department of Faculty of Pharmacy- AL -Azhar university-Cairo using Mueller-Hinton agar (MHA) assay (Mansour-Ghanaei et al., 2022), where a concentration of 1000 µg/ml (w/v) was prepared for each tested fraction using DMSO at concentration of 50%. Standardized concentrations (McFarland were that corresponding to 1.5×10^8 CFU) of cultures of the test bacterial isolates was prepared and swabbed aseptically on the agar plates and holes (6 mm) using a sterile cork borer. 50 µl of each fraction and controls were inoculated in each hole under aseptic condition, kept at room temperature for 1 h to allow the agents to

diffuse into the agar medium and then incubated. Clarithromycin (100 µg/ml) positive control for bacteria, while DMSO (50%), used as the negative control. The MHA plates were then incubated and inverted at 37°C for 48 hours under microaerophilic condition using candle jar. The inhibition zone diameters were measured in millimeters. The MIC values were determined using broth microdilution method by polystyrene microtiter plates with 96 wells (Oy Growth Curves Ab Ltd., Helsinki, Finland), using Muller-Hinton broth as indicated by the Clinical and Laboratory Standards Institute of the United States of America (CLSI, 2020) (Huang et al., 2021), where a stock concentration of the tested samples was employed. Two-fold serial dilution was conducted. The wells were filled with a total volume of 200 µl, made up of 20 µl of the antimicrobial solution at a range of concentrations and 170 µl of Muller-Hinton broth and 10 µl of the tested strain at concentration of 1.5×10^8 CFU. Negative controls with 100 µl of Muller-Hinton broth and 100 µl of the tested compounds and positive controls with 200 µl of the bacterial inoculum were used. Growth was determined by adding 50 µl of Resazurin 0.01%. The DCM and EtOAc fractions exhibited the highest antimicrobial activities so they were subjected to different chromatographic techniques in order to obtain the compound/s responsible for their activities. The DCM fraction (3g) was chromatographed over a normal-phase silica gel column (2×50 cm) using DCM/MeOH (100:0 → 90:10, v/v) to give 5 sub fractions (D1-D5). Subfraction D2 (98:2 DCM/MeOH, 0.2 g) was purified over Sephadex LH-20 column (2×60 cm, 100% MeOH) to afford 25 mg of the pure compound **(1)**. The EtOAc fraction (15g) was chromatographed using silica gel (normal phase) column chromatography (5×50 cm) using DCM-MeOH (100%-70:30 v/v) to give 5 sub fractions (E1-E5). Sub fraction E2 (3.6g) was further chromatographed on silica gel column (2×60 cm) chromatography (normal phase) using DCM and MeOH (100:0–90:10 v/v) to give 6 sub fractions (E2a-E2f), then the sub fraction E2e (0.4g, eluted with DCM: MeOH 90:10 v/v) was purified on sephadex LH-20 column (2× 60 cm, 100% MeOH) to afford 30 mg of compound **(2)**. Sub fraction E3 (4.5g) was further chromatographed on silica gel column (2×60 cm) chromatography (normal phase) using DCM and MeOH (100:0–90:10 v/v) with to give 6 sub fractions (E3a-E3f), then the sub fraction E3f (0.5g, eluted with DCM: MeOH 90:10) was purified on sephadex LH-20 column (2×60 cm, 100% MeOH) to afford 60 mg of compound **(3)**. Sub fraction E5 (1.1g) was further chromatographed on silica gel column (2×60 cm) chromatography (normal phase) using DCM and MeOH (90:10–75:25 v/v) to give 4 sub fractions (E5a-E5d), then the sub fraction E5d (0.1g, eluted with DCM: MeOH 75:25 v/v) was purified on sephadex LH-20 column (2× 60 cm, 100% MeOH) to afford 10 mg of compound **(4)**.

Results and Discussion

Compound **1** was obtained as colorless crystalline solid; its molecular formula was determined as $C_{14}H_{22}O_3$ through HR-ESI-MS (qTOF) which showed a molecular ion peak at m/z 283.1551 $[M+FA-H]^+$. The UV spectrum of **1** showed one absorption maximum at λ 253 nm. The ^1H NMR spectrum (Table 1) of compound **1** showed the presence of two aromatic *ortho*-coupled protons at δ 6.07 (d, J = 9.8 Hz, 1H) and 6.84 (d, J = 9.8 Hz, 1H), four methyl proton signals at δ 0.92 (d, J = 6.7 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.38 (s, 3H), and 1.86 (s, 3H). Additionally, the ^1H NMR spectrum revealed the presence of two methylene proton signals equivalent to four protons at δ 0.83 (dd, J = 7.4, 3.8 Hz, 2H), 1.17 (m, 2H), three

methines at δ 1.23, 2.16, and 4.90. The ^{13}C NMR spectrum of **1** (Table 1) showed the presence of one carbonyl at δ 186.7 ppm, as well as four aromatic carbons at δ 124.7, 129.2, 159.1, and 160.2, suggesting the presence of pyrone ring. The appearance of two aromatic *ortho*-coupled protons in the ^1H NMR, suggested a disubstituted pyrone ring. Moreover, the ^{13}C NMR spectrum showed four methyl carbons resonances at δ 10.42, 21.07, 21.59, 25.63 ppm, one aliphatic oxygenated carbon at δ 67.40 ppm, two methylene carbons at δ 37.81, 49.99 ppm and two aliphatic methines at δ 28.74 and 40.02 ppm, suggesting the presence of an aliphatic hydroxylated side chain attached to the pyrone moiety. The γ -pyrone moiety was deduced from the presence of four aromatic carbons and one carbonyl carbon, in addition to the UV absorption maxima at λ 253 nm compared to that of α -pyrone at λ max 300 nm (Soliman *et al.*, 2007). Further, the HSQC experiment was in a line with the suggestion of a γ -pyrone with an aliphatic side chain. Furthermore, the HMBC correlations (Figure 1) confirmed the structure of **1** to be γ -pyrone with a methyl group at position C3 and an aliphatic side chain at position C2. The HMBC correlations between δ_{H} 6.07 / δ_{C} 159.14 and 186.72, and δ_{H} 6.8 and δ_{C} 186.7 and 160.2, further confirmed the presence of γ -pyrone. The attachment of methyl group at C3 position of γ -pyrone ring was confirmed by correlations between the methyl protons at δ 1.86 and carbon at δ 129.23 (C-3) in HMBC spectrum. The skeleton of aliphatic side chain was confirmed through the correlations between the two terminal methyl protons at δ 0.92 and 0.96 with carbons at 28.7 and 49.99, correlation between proton signal at δ 0.83 and carbon at δ 67.40 ppm, correlation between proton signal at δ 4.9 and carbon at δ 37.8, and correlation between proton signal at δ 1.38 and carbons at δ 37.8 and 40.02. The attachment of aliphatic side chain at C-2 of the γ -pyrone ring was confirmed through the correlations of proton at δ 1.38 with carbon at 160.23 (C-2 of γ -pyrone ring). Based on the above mentioned data, compound **1** was identified as 2-(4-hydroxy-6-methylheptan-2-yl)-3-methyl-4*H*-pyran-4-one and named as Kadipyrone, which is in a good agreement with related γ -pyrone derivatives in literature (Shin *et al.*, 1986, Wilk *et al.*, 2009, Labuda *et al.*, 2021). This is the first report of this compound in nature.

Compound **2** was obtained as light yellow powder, ^1H NMR (400 MHz, DMSO- d_6) δ 6.67 (s, H3), 6.19 (d, J = 1.9 Hz, H6), 6.45 (d, J = 1.9 Hz, H8), 7.42 (d, J = 1.9 Hz, H2'), 6.89 (d, J = 8 Hz, H5), 7.40 $p \pm$ (H – 6 overlapped with H2'). ^{13}C NMR (100 MHz, DMSO- d_6) δ 163.95 (C2), 102.86 (C3), 181.76 (C4), 161.51 (C5), 98.94 (C6), 164.39 (C7), 93.96 (C8), 157.36 (C9), 103.68 (C10), 121.47 (C1), 113.38 (C2), 145.85 (C3), 149.87 (C4), 116.11 (C5), 119.04 (C6). ESI/MS: m/z 285 $[\text{M}-\text{H}]^-$ (calc. for $\text{C}_{15}\text{H}_{10}\text{O}_6$). Compound **2** was identified as luteolin (Tawornchat *et al.*, 2021).

Compound **3** was obtained as yellow powder, ^1H NMR (400 MHz, DMSO- d_6) δ 6.19 (d, J = 1.7 Hz, H6), 6.41 (d, J = 1.6 Hz, H8), 7.68 (d, J = 2.0 Hz, H2'), 6.88 (d, J = 8.5 Hz, H5'), 7.54 (dd, J = 8.5, 2.0 Hz, H6'). ^{13}C NMR (100 MHz, DMSO- d_6) δ 147.24 (C2), 136.18 (C3), 176.29 (C4), 161.17 (C5), 98.64 (C6), 164.38 (C7), 93.81 (C8), 156.59 (C9), 103.45 (C10), 122.41 (C1), 115.51 (C2), 145.51 (C3), 148.15 (C4), 116.06 (C5), 120.42 (C6). ESI/MS: m/z 303 $[\text{M}+\text{H}]^+$, 301 $[\text{M}+\text{H}]^-$ (calc. for $\text{C}_{15}\text{H}_{10}\text{O}_7$). Compound **3** was identified as quercetin (Sambandam *et al.*, 2016).

Compound **4** was obtained as white crystalline powder, ^1H NMR (400 MHz, DMSO- d_6) δ 7.33 (d, $J=8.9$ 2H, H2 & H6), 6.67 (d, $J=8.8$ 2H, H3 & H5). ^{13}C NMR (100 MHz, DMSO- d_6) δ 124.99 (C1), 130.37 (C2,C6), 115.00 (C3,C5), 160.07 (C4), 171.55 (COOH). ESI/MS m/z 139 $[\text{M}+\text{H}]^+$ (calc. for $\text{C}_7\text{H}_6\text{O}$). Compound **4** was identified as Para hydroxy benzoic acid (CHO *et al.*, 1998).

The results of antifungal assays (Table 2) showed that DCM and EtOAc fractions have good antifungal action against *Candida tropicalis* with MIC values of 1250 and 2500 $\mu\text{g/ml}$, respectively. Conversely, all fractions (*n*-Hexane, DCM, EtOAc and *n*-BuOH) are inactive against *Aspergillus fumigatus*. Evaluation of the antibacterial activity (Table 2) showed that the DCM and EtOAc fractions exhibited good antibacterial activities against *Staphylococcus epidermidis* with MIC values of 625 and 1250 $\mu\text{g/ml}$, respectively, and against *Salmonella typhimurium* with MIC values of 2500 and 3500 $\mu\text{g/ml}$, respectively. Moreover, the results of anti *H.pylori* activity indicated that the DCM fraction of *H. sanguineum* has the highest activity with MIC value of 64 $\mu\text{g/ml}$ as depicted in Table 3 and Figure 2. Importantly, compound **1**, isolated from DCM fraction, was also tested against *H. pylori*, and the results displayed that compound **1** possess a potent antibacterial activity against *H. pylori* with MIC value of 32 $\mu\text{g/ml}$. In conclusion

Declarations

Acknowledgements

The authors are grateful to Mohammed Salah Abdulrahman, Lecturer of microbiology and immunology faculty of pharmacy Alazhar University for conducting the *anti-H.pylori* assays.

Authorship

Adel M Mahdi: Plant collection, extraction, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Taha A Kedra**: original manuscript writing, data curation. **Muhamad Abd-Elraouf**: analysis of data, methodology, writing and revision. **Usama Shaheen**: analysis of data, supervision, data curation. **Ezzat A. M. Genady**: Writing & editing, Supervision, data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

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Tables

Table 1: ^1H NMR (400 MHz, DMSO-d₆), ^{13}C NMR (100 MHz, DMSO-d₆) and HMBC of the compound (1).

Position	$\delta^{13}\text{C}$	$\delta^1\text{H}$ (J in Hz)	HMBC
2	160.23		
3	129.23		
4	186.72		
5	124.73	6.07 (d, J = 9.8 Hz, 1H)	159.14, 186.72
6	159.14	6.84 (d, J = 9.8 Hz, 1H)	160.23, 186.72
7	40.02	2.16 (d, J = 5.0 Hz, 1H)	
8	37.81	1.17 (m, 2H)	67.40
9	67.40	4.90 (m, 1H)	37.81, 49.99
10	49.99	0.83 (dd, J = 7.4, 3.8 Hz, 2H)	67.40
11	28.74	1.23 (s, 1H)	
12	21.59	0.92 (d, J = 6.7 Hz, 3H)	21.07, 28.74, 49.99
13	10.42	1.86 (s, 3H)	129.23, 160.23, 186.72
14	25.63	1.38 (s, 3H),	37.81, 40.02, 160.23
15	21.07	0.96 (d, J = 6.6 Hz, 3H)	21.59, 28.74, 49.99

J -values are reported in Hz; chemical shifts are given in ppm; assignments were confirmed by HSQC, and HMBC experiments. Data measured in dms_o-d₆.

Table 2: Antimicrobial activity of *H. sanguineum* fractions (zone of inhibition and MIC).

	DCM		EtOAc		<i>n</i> -BuOH		<i>n</i> -Hex	Control
-	Inhibition zone	MIC	Inhibition zone	MIC	Inhibition zone	Inhibition zone	Inhibition zone	Inhibition zone
<i>Aspergillus Fumigatus</i>	NA	-	NA	-	NA	NA	NA	17
<i>Candida Tropicalis</i>	23	1250	19	2500	15	NA	NA	21
<i>Staphylococcus epidermidis</i>	22	625	15	1250	12	10	10	28
<i>Bacillus subtilis</i>	16	-	13	-	11	NA	NA	26
<i>Salmonella typhimurium</i>	20	2500	15	3500	14	NA	NA	17
<i>Serratia marcescens</i>	19	-	13	-	12	NA	NA	25

Positive control for fungi (Ketoconazole 100 µg/ml), Positive control for bacteria (Gentamycin 4 mg/ml), *NA: No activity. The sample was tested at 10 mg/ml concentration. Zone of inhibition was measured in mm. MIC was measured in µg/ml.

Table 3: Anti *H. Pylori* activity (well diffusion assay) of *H. sanguineum* DCM fraction and compound **1** and their MICs.

Samples	Well diffusion assay					MIC ($\mu\text{g /ml}$)
	Plate -1	Plate-2	Plate-3	Mean	SD ($\pm$)	
Compound 1	34	33	32	33	0.816497	32
DCM	31	30	31	30.66667	0.471405	64
Clarithromycin	24	22	23	23	0.816497	128
DMSO 50%	12	10	10	10.66667	0.942809	-

Zone of inhibition is measured in mm, clarithromycin is positive control, and DMSO is negative control. MIC is expressed in ($\mu\text{g /ml}$).

Figures

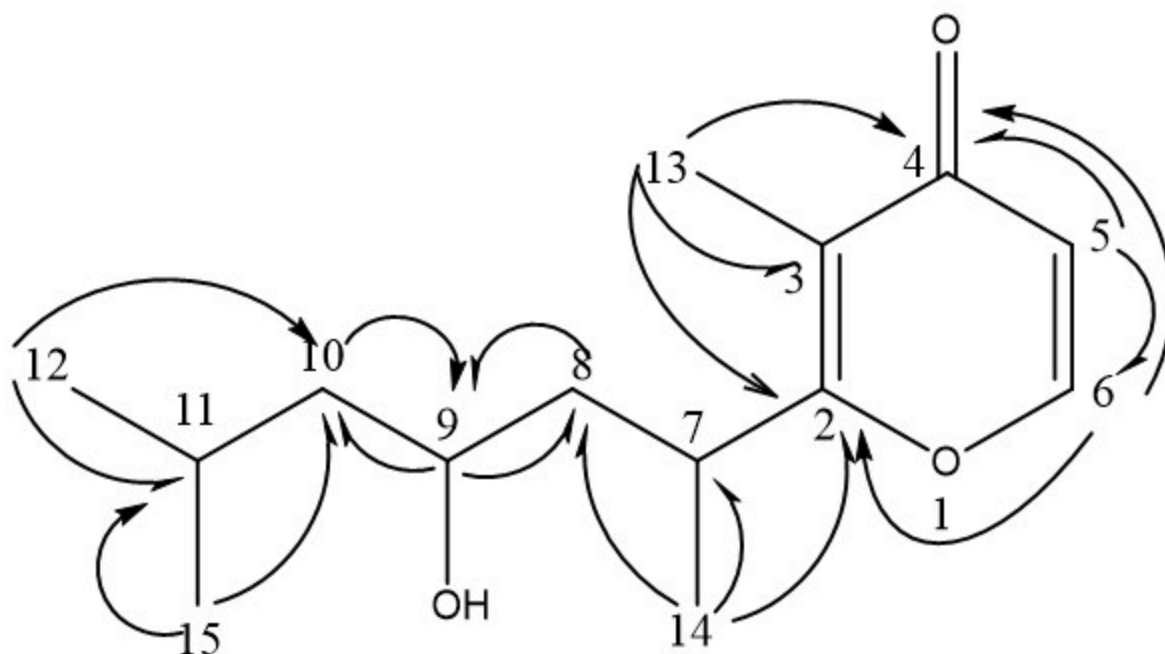


Figure 1

Key HMBC correlations of compound **1** (DMSO-d₆)

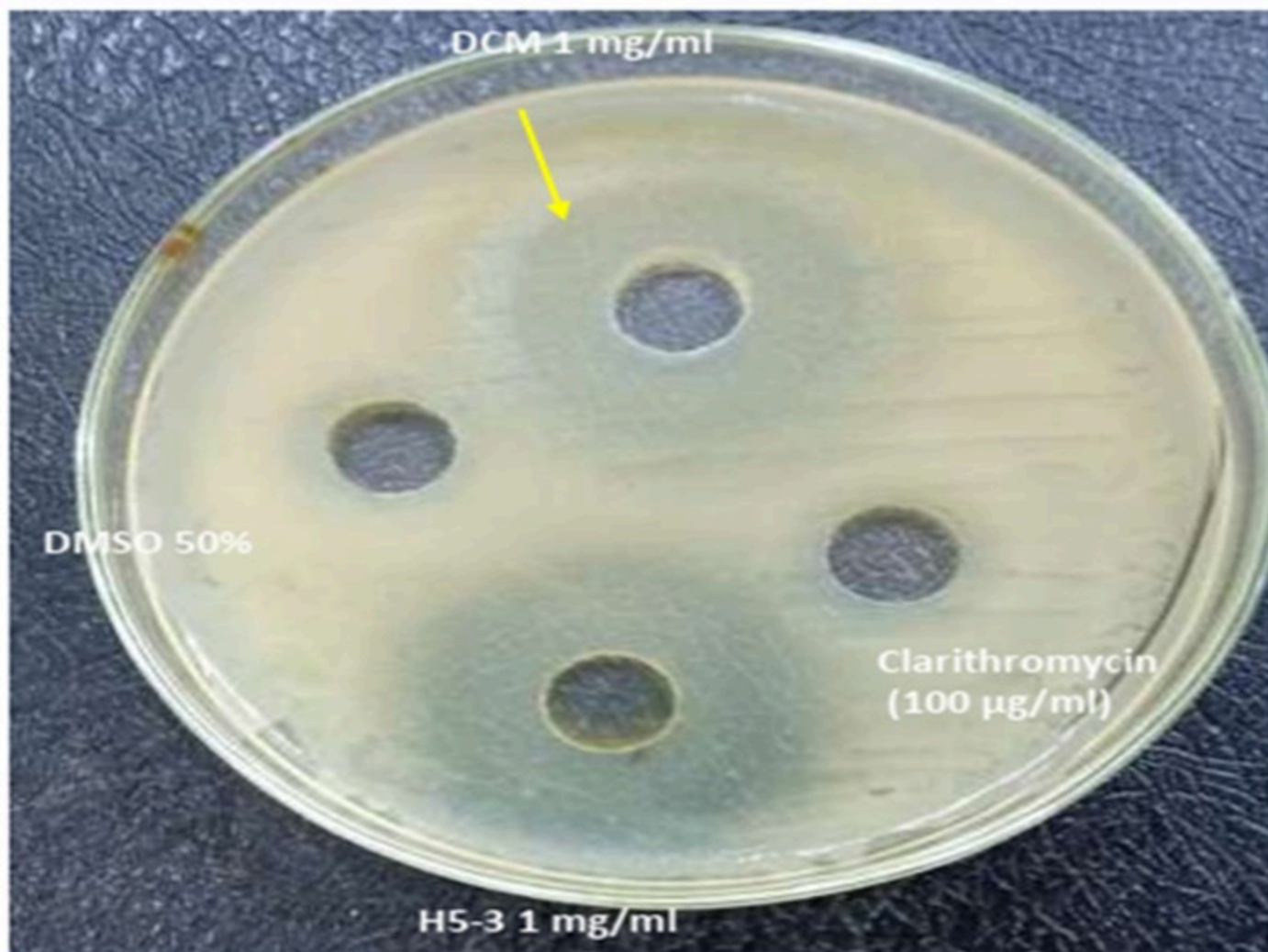


Figure 2

Evaluation of Anti-H-Pylori activity (well diffusion) of DCM fraction and compound **1**

N.B: HS-3 is compound **1**

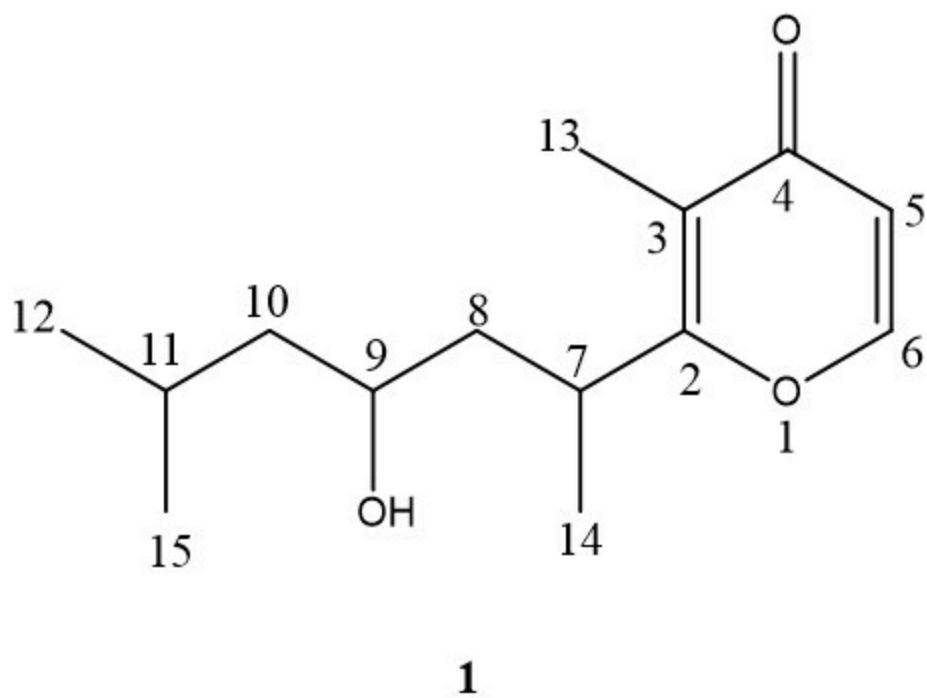


Figure 3

Unnumbered image in the Results and Discussion section.

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