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Anti-inflammatory and antimicrobial activities of the successive extracts of the aerial parts of *Rumex pictus* Forssk. growing in Egypt

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ABSTRACT

Introduction: *Rumex* spp. have been used in folk medicine either as food or as medicine for the treatment of several diseases including constipation, fever, inflammation, bacterial and fungal infections. This study aimed to evaluate the anti-inflammatory and antimicrobial activities of the successive extracts of the aerial parts of *Rumex pictus* Forssk. growing in Egypt, and to identify the chemical constituents in the bioactive extract.
Methods: Ether, chloroformic, and 70% methanolic extracts of the aerial parts of *R. pictus* were assayed for their *in vivo* anti-inflammatory activity using carrageenan-induced rat hind paw edema method. These extracts were also tested for their *in vitro* antibacterial and antifungal activities using disc diffusion method.
Results: The 70% methanolic extract of *R. pictus* exhibited significant anti-inflammatory, antibacterial, and anti-candida activities. Thus, fractionation of the bioactive extract was performed which led to the isolation of three anthraquinones, as well as, seven flavonoids.
Conclusion: *Rumex pictus* possesses anti-inflammatory and antimicrobial activities which reinforce its use in ethnomedicine.

Implication for health policy/practice/research/medical education:
Rumex pictus showed significant anti-inflammatory and antimicrobial activities thus might be used as a complementary medicinal plant for treating inflammation and infection after further clinical studies.
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Introduction

The genus *Rumex*, is the second largest genus of family Polygonaceae with around 200 species distributed worldwide, mainly in the Northern hemisphere (1). Plants from the genus *Rumex* have been used in folk medicine either as food or as medicine for the treatment of several diseases including constipation, stomach disorders, fever, inflammation, bacterial and fungal infections and rheumatism. *Rumex* spp. have been marked by the presence of phenolic compounds including anthraquinones, naphthalene-1,8-diols, flavonoids and stilbenoids (1). Chrysophanol, emodin and physcion appear to be the most common anthraquinones reported in *Rumex* spp (2). Flavonoids reported in *Rumex* include flavonols, flavan-3-ols, O- and C-glycosides (3). *Rumex pictus* Forssk., commonly known as veined dock is an annual herb that grows wild in Egypt, Syria and Arabia. *R. pictus* is locally known as hummayd, hammad (sour-wort), khamsees and khansees. It is an edible plant, collected in Spring and eaten fresh or cooked. Previous studies indicated that other *Rumex* spp. as *R. obtusifolius, R. nervosus, R. abyssinicus, R. crispus* and *R. patientia* significantly exhibited anti-inflammatory and antimicrobial activities (4-7). Phytochemical

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investigation of *R. pictus* Forssk. revealed the presence of anthraquinones, flavonoids, volatiles, carbohydrates and/or glycosides, sterols and /or terpenes, amino acids and tannins, in addition to, significant cytotoxic activity of the various successive extracts with different proportions (8). Moreover, flavonoids (luteolin, apigenin, apigenin 7-O-*β*-*D*-glucoside, quercetin 3-*O*-*β*-*D*-glucuronide, quercetin-3O-{2'''-acetyl-α-L-arabinopyranosyl-(1→6)-β-D-galacto-pyranosyl}, quercetin-3O-arabinosyl galactoside, kaempferol-3O-{2'''-acetyl-α-L-arabinopyranosyl-(1→6)-β-D-galactopyranosyl}, kaempferol-3O-arabinosyl galactoside, orientin, isoorientin, vitexin and isovitexin), as well as, anthraquinone (chrysophanol) and a new 8-ionized hydroxylated 9,10-anthraquinone namely, 1-hydroxy-3-methyl-9,10-anthraquinone-6-O-β-D-glucopyranoside-8-olate (rumpictuside A) were isolated from *R. pictus* (2,9,10). The objective of this study was to evaluate the anti-inflammatory and antimicrobial activities of the successive extracts of *R. pictus* Forssk. growing in Egypt, as well as fractionation and identification of the chemical constituents in the bioactive extracts.

Materials and Methods

Plant material

The aerial parts of *R. pictus* Forssk. were collected from North of Al-Manzala Lake (Damietta-Port Said road), Egypt. The plant was authenticated by Dr. Abd El-Haleem Abd El-Motagaly, Department of Flora, Agricultural Museum, Giza, Egypt. Voucher specimens (RP 201) were deposited at the herbarium of the National Research Centre, Giza, Egypt. The collected plants under investigation were air dried, powdered and reduced to mesh no. 36 and kept in tightly sealed containers.

The phytochemical study

Preparation of successive extracts with selective organic solvents

Ground air-dried aerial parts of *R. pictus* (500 g) were extracted in a Soxhlet continuous extraction apparatus successively and exhaustively using solvents of increasing polarity in the following order: petroleum ether (60-80°C), diethyl ether, chloroform and 70% aqueous methanol. For each solvent, extraction was continued to exhaustion. In each case, the solvent was stripped off by distillation under reduced pressure at a temperature not exceeding 40°C and dried at a constant weight in a vacuum desiccator over anhydrous calcium chloride. Successive extracts were then chromatographed on thin layer chromatography (TLC) and paper chromatography. Similar extracts in chemical composition with different proportions were combined together resulting in three major extracts; total ether extract (TE) consisted of petroleum ether and diethyl ether extracts, chloroformic extract (CE) and 70% methanolic extract (ME).

Quantitative estimation of total phenolic content

Total phenolic content was determined by applying a modified Folin-Ciocalteu method (11,12). 20 mg of the 70% aqueous methanolic extract was dissolved in 25 mL of distilled water. The solution (0.5 mL) was then mixed with 5 mL of 7.5% Na_2CO_3 solution and 5 mL Folin-Ciocalteau reagent (1:10). The mixture was then left in the dark for 30 minutes and measured spectrophotometrically at 745 nm. The total phenolic content was expressed as milligrams of gallic acid equivalent (GAE) per g of dried sample. All measurements were conducted in triplicates.

Quantitative estimation of total flavonoid content

Total flavonoid content was determined in the 70% aqueous methanolic extract of *R. pictus* as described by Rolim et al (13). The dry aqueous methanolic extract was dissolved in ethanol: water (20:80) (v/v) in an amount of 2 mg/mL. The solution (0.5 mL) was mixed with 5 mL of ethanol 95%: acetic acid 96% (99:1) (v/v) and measured spectrophotometrically at 362.8 nm. The total flavonoid content was expressed as milligrams of rutin equivalent (RE) per g of dried sample. All measurements were conducted in triplicates.

Investigation of the bioactive 70% methanolic extract

The methanolic extract was concentrated to give a residue (45 g), which was applied to a polyamide 6S (Riedel de Haen, Germany) column and eluted using water with proportional increasing of methanol. All fractions obtained were combined according to their paper chromatographic analysis to give two major fractions (**I** and **II**). Each fraction was separately subjected to reversed phase (RP C-18) (Sigma-Aldrich, USA) column Chromatography (RP C-18) using methanol with proportional increasing of water as eluent. Compound **1** (8 mg), compound **2** (10 mg) and compound **3** (200 mg) were obtained as major compounds from fraction **I**. Fraction **II** yielded compound **4** (10 mg), compound **5** (12 mg), compound **6** (25 mg), compound **7** (18 mg), compound **8** (20 mg), compound **9** (7 mg) and compound **10** (8 mg). Each compound was separately purified by repeated elution on Sephadex LH-20 (Pharmacia Fine Chemicals, Sweden) using methanol. Compounds **3**-**8** (2 mg) were subjected to acid hydrolysis (14) in a mixture of 8% HCl (1 ml) and MeOH (4 ml) were separately refluxed for 2 hours. The reaction mixtures were reduced under pressure to dryness, dissolved in H_2O (3 ml) and neutralized with Na_2CO_3. The neutralized products were subjected to paper chromatography using eluent (benzene: *n*-butanol: pyridine: H2O, (1:5:3:3) (v/v/v/v)). The chromatograms were detected with aniline hydrogen phthalate followed by heating at 100°C. Compounds **7**-**8** released no sugar, thus indicating the presence of *C*-glycosylation. The sugars of compounds **3**-**6** were identified after comparison with authentic samples.

Spectroscopic procedures

Nuclear magnetic resonance (NMR) spectra were obtained on Bruker 400 (1H, 400 MHz; 13C, 100 MHz). DMSO-d6 was used as solvent. The δ values reported as ppm relative to TMS in DMSO-d6 and J values are given in Hz. Ultraviolet (UV) spectra were recorded on Shimadzu UV-visible spectrophotometer model-UV 240 (Tokyo, Japan).

The biological study

The plant extracts used were the total ether extract (TE), chloroformic extract (CE) and 70% methanolic extract (ME) prepared from the aerial parts of *R. pictus*.

Anti-inflammatory activity (carrageenan-induced rat hind paw edema assay)

Adult rats of both sexes weighing 150-200 g were used in the experiments. Animals were housed under standardized conditions of light and temperature and received standard rat chow and tap water *ad libitum*. Animals were randomly assigned to different experimental groups, each kept in separate cage. Twenty-four adult male albino rats, divided into four groups, each of six animals, were orally treated with different extracts (TE, CE, ME) (300 mg/kg), indomethacin (Kahira Pharmaceutical and Chemical Company, Egypt) (20 mg/kg) (positive control), and saline (negative control). One hour after oral administration, all animals were given a sub-plantar injection of 100 µL of 1% carrageenan (Sigma Aldrich, USA) solution in saline 100 mL in the right hind paw. The contra-lateral hind paw received the same volume of saline and served as normal control. Hind foot-pad thickness was measured with a micrometer caliber (15) before and at 1, 2, 3 and 4 hours after carrageenan injection, as carrageenan caused visible redness and pronounced swelling that was well developed in 4 hours and persisted for more than 48 hours (16).

Antimicrobial activity (disc diffusion method)

Tested microorganisms including two gram-positive bacterial strains (*Bacillus cereus* and *Staphylococcus aureus* ATCC 6538), three Gram-negative bacterial strains (*Escherichia coli* NRRN 3008, *Salmonella typhimurium* ATCC 25566 and *Pseudomonas aeruginosa* ATCC 10145), three fungi (*Mucor miehei* NRRL 2034 and *Aspergillus niger* NRRL 595) and one yeast (*Candida albicans* EMCC 105) were used for this experiment. The strains were kindly provided by the Chemistry of Natural and Microbial Products Laboratory, Chemistry of Natural and Microbial Department, NRC, Cairo, Egypt. Nutrient agar medium, potato- dextrose agar growth medium (PDA) and yeast extract peptone dextrose (YEPD) medium were used. All chemicals used in the preparation of the media were of the analytical grade. Distilled water was used. Routine sterilization was done by autoclaving for 20 minutes at 15 psi (121°C). The prepared extracts (TE, CE, ME) were assessed for their antimicrobial activity against Gram-positive and Gram-negative bacteria, fungi and yeast, using the disc diffusion method (17). A sterilized filter paper disc saturated with measured quantity (20 µL) of the sample (1 mg/mL) was placed on a plate of 10 cm diameter containing a solid bacterial, fungal or yeast medium which has been seeded with the spore suspension of the test organism. After incubation (at 37°C for 24 hours for bacteria, at 25°C for 72 hours in case of fungi, and at 28°C for 24 hours for the yeast), the diameter of the inhibition zone surrounding the sample was taken as a measure of the inhibitory power of the sample against the particular test organism. Ampicillin (Wyeth) (20 µL) was used as reference antibacterial agent while clotrimazole (Bayer) (20 µL) was used as a reference antifungal drug. All these steps were carried out under aseptic conditions.

Statistical analysis

All values were expressed as mean ± standard error (SE). All results were statistically evaluated and $P<0.05$ was considered statistically significant. Methods of statistical analysis were performed according to (18).

Results

Anti-inflammatory activity

The sub-plantar administration of 100 µL of 1% sterile carrageenan into the rat hind paw provoked an inflammation (swelling and erythema) and a time-dependent increase in paw edema that was highest at 4 hours post carrageenan. All of the tested extracts showed inhibition of the induced inflammation with varying percentages during all phases of inflammation in this model (Figure 1). The 70% methanolic extract of *R. pictus* aerial parts (300 mg/kg) showed the highest inhibition of edema formation by 68.97%, 55.56%, 57.89% and 57.89% at 1, 2, 3 and 4 hours (post carrageenan injection), respectively when compared with control group. The total ether and chloroformic extracts of *R. pictus* aerial parts (300 mg/kg) showed equal inhibition of the edema formation 34.21% at 4 hours (carrageenan injection), as compared with control group.

Antimicrobial activity

The 70% aqueous methanolic extract of *R. pictus* exhibited significant antibacterial activities against gram-negative *E. coli*, *P. aeruginosa* and *S. typhimurium* (66.7%, 60% and 80%, respectively, of ampicillin inhibitory power) and against gram-positive *B. cereus* and *Staph. aureus* (40% and 36.6%, respectively, of ampicillin inhibitory power) (Table 1). Moreover, this extract exhibited high activity against *C. albicans* (40% of clotrimazole inhibitory power). Whereas, the total ether extract elicited significant antibacterial activity only against gram-positive *B. cereus* and *Staph. aureus* (51% and 60%, respectively, of ampicillin inhibitory power). Chloroformic extract inhibited the

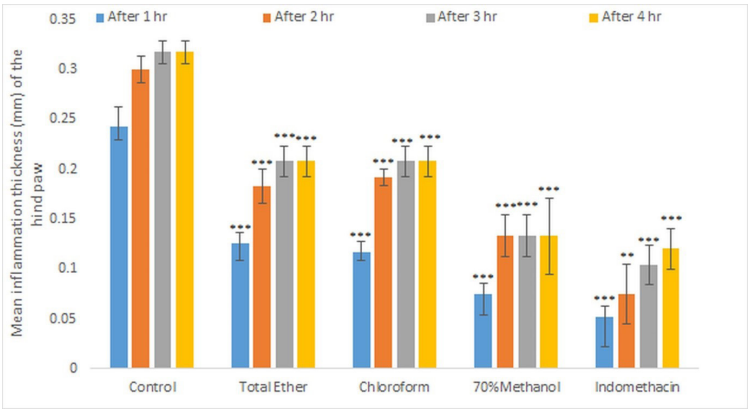


Figure 1. Mean inflammation thickness (mm) of the hind paw of control and tested rats at different time intervals of carrageenan injection. ***P*<0.005, ****P*<0.001 in comparison with control group according to student's *t* test.

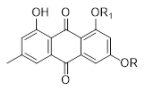
growth of *B. cereus* (28.5% of ampicillin inhibitory power), as well as, *C. albicans* (32.5% of clotrimazole inhibitory power). On the other hand, none of the tested extracts showed any antifungal activity against *M. miehei* and *A. niger*.

The phytochemical study

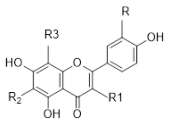
The 70% aqueous methanolic extract of *R. pictus* revealed significant suppression of carrageenan induced swelling of the hind rat paw by 68.97%, 55.56%, 57.89% and 57.89% at 1, 2, 3 and 4 hours. Moreover, this extract showed significant antibacterial and anticandidal activities. Hence, it was necessary to investigate the chemical composition of this extract to stand for the constituents which may be responsible for its biological activity. Total phenolic and total flavonoid contents of the bioactive extract were assayed spectrophotometrically and were calculated as 12.752 mg (GAE)/g and 3.931 mg (RE)/g, respectively. Furthermore, fractionation using several chromatographic techniques (column, paper and TLC) of the bioactive extract resulted in ten pure compounds, most of which were separated first-ever from this species (Figure 2). They were specified by different spectral techniques UV and (^{1}H- and ^{13}C) NMR also by co-chromatography against standard sugars and authentic aglycones after complete acid hydrolysis, as well as reported literature (19,20). The presence of *peri*-hydroxy anthraquinones in compounds **1**-**3** were detected by their red color with 5% KOH solution on TLC. ^{1}H NMR spectrum revealed the 9, 10 anthraquinones with the presence of chelated hydroxyl groups. Compounds **4**-**10** appeared as dark purple spots on PC under UV light, changing to yellow when exposed to ammonia vapor, indicating a flavonoid structure. Diagnostic UV shifts of the compounds **4**-**6** were identical with 5-hydroxy-3-substituted flavonols. Furthermore, the ^{1}H NMR spectra showed the signals of A- and B-ring. However, a characteristic carbon resonance at δ 172.2 for C-6″ of the glucuronic acid moiety of **6** was observed. On the other hand, UV spectral properties of compounds **7**-**10** were in accordance with flavone glycosides (**7** and

Table 1. Antimicrobial activities of successive extracts of the aerial parts of *Rumex pictus* via disc diffusion method against selected pathogenic isolates

Pathogenic microbial isolates	Total Ether	Chloroform	70% Methanol	Ampicillin	Clotrimazole
	Mean inhibition zone diameter (mm) ± S.D (20 µL)				
Gram-positive bacteria					
Bacillus cereus	18±0.21	10±0.1	14±0.2	35±0.11	0
Staph. aureus	18±0.1	0	11±0.11	30±0.3	0
Gram-negative bacteria					
E. coli	0	0	10±0.15	15±0.21	0
P. aeruginosa	0	0	12±0.15	20±0.11	0
S. typhimurium	0	0	12±0.3	15±0.25	0
Fungi					
Mucor miehei	0	0	0	0	25±0.25
A. niger	0	0	0	0	25±0.27
Yeast					
C. albicans	0	13±0.15	16±0.21	0	40±0.17



1 R=R1=H
2 R=CH3; R1=H
3 R=H; R1= β-D-glucose



6 R=OH; R1= O-β-D-glucuronic acid; R3=R4=H
7 R=OH; R1= R2=H; R3=β-D-glucose
8 R=OH; R1= R3=H; R2=β-D-glucose
9 R= R1=R2=R3=H
10 R=OH; R1=R2=R3=H

Figure 2. Structures of compounds 1-10.

8) having free 5-,7- and 4′-hydroxyl groups and flavone aglycones (**9** and **10**). By acid hydrolysis of the original compounds (**7** and **8**), another flavonoid was produced by Wessely-Moser rearrangement, showing the original flavonoid was 6- or 8-*C*-glycosylflavone.

Chemical characterization of isolated compounds

Emodin (1)

Orange crystals, TLC 0.35 (5% CH_3OH/CH_2Cl_2). UV λ_{max} (MeOH): 225, 258, 291, 429. ^{1}H NMR: δ = 12.2 (s, 1H, OH-1), 12.11 (s, 1H, OH-8), 7.7 (d, *J* = 2 Hz, H-4), 7.26 (d, *J* = 2 Hz, H-5),7.08 (d, *J* = 2 Hz, H-7, H-2), 6.67 (d, *J* = 2 Hz, H-7), 2.35 (s, 3H, -CH_3).

Physcion (1,8-Dihydroxy-3-methoxy-6-methyl- 9,10-anthraquinone) (2)

Orange crystals, TLC 0.38 (5% CH_3OH/CH_2Cl_2). UV λ_{max} (MeOH): 255, 270, 385, 424. ^{1}H NMR: δ = 12.34 (s, 1H, OH-1), 12.14 (s, 1H, OH-8), 7.63 (s, H-4), 7.37 (d, *J* = 2 Hz, H-5), 7.08 (s, H-2), 6.69 (d, *J* = 2 Hz, H-7), 3.93 (s, -OCH_3), 2.44 (s, 3H, -CH_3).

Emodin-8-O-β-D-glucoside (3)

Orange powder, TLC 0.2 (5% CH_3OH/CH_2Cl_2). UV λ_{max} (MeOH): 219, 247, 282, 415. ^{1}H NMR: δ = 13 (s, 1H, OH-1), 7.45 (br, 1H, H-4), 7.2 (d, *J* = 2Hz, H-5),7.15 (br, 1H, H-2), 6.90 (d, *J* = 2 Hz, H-7), 4.99 (H-1'), 2.39 (s, -CH_3).^{13}C NMR: δ = 186.27 (C-9), 182.99 (C-10),164.51 (C-6),162.18 (C-1),161.97 (C-8),146.95 (C-3), 136.30 (C-10a), 132.09 (C-4a), 124.56 (C-2), 119.54 (C-4), 115.09 (C-9a), 112.10 (C-8a),110.34 (C-7),109.39 (C-5),101.65 (C-1'), 77.77 (C-5'),76.76 (C-3'),73.77 (C-2'), 69.93 (C-4'), 61.03 (C-6'), 21.73 (-CH_3).

Astragalin (kaempferol 3-O-β-glucopyranoside) (4)

Yellow powder, PC R_f 0.57 (butanol: acetic acid: water (4:1:5)) (BAW), 0.42 (15% acetic acid (HOAc)). UV λ_{max} (MeOH): 267, 327sh, 352; (NaOMe): 274, 325, 380; ($AlCl_3$): 277, 301, 350, 406; ($AlCl_3$/HCl): 274, 303, 350, 400; (NaOAc): 271, 304, 371; (NaOAc/H_3BO_3): 266, 305, 350. ^{1}H NMR: δ = 12.41 (s, 1H, -OH), 8.00 (dd, *J* = 7.64 & 2 Hz, H-2'/6'), 6.80 (dd, *J* = 7.68 & 2 Hz, H-3'/5'), 6.25 (d, *J* = 2 Hz, H-8), 6.04 (d, *J* = 2 Hz, H-6), 5.29 (d, *J* = 7.5Hz, H-1").

Isoquercitrin (quercetin 3-O-β-glucopyranoside) (5)

Yellow powder, PC R_f 0.47 (BAW), 0.45 (15% HOAc). UV λ_{max} (MeOH): 257, 301, 324sh, 353; (NaOMe):273, 315, 395; ($AlCl_3$): 268, 275, 301, 434; ($AlCl_3$/HCl): 268, 275, 300, 356; (NaOAc): 264, 301, 386; (NaOAc/H_3BO_3): 264, 301, 371. ^{1}H NMR: δ = 12.46 (s, 1H, -OH), 7.52 (d, *J* = 7.84 Hz, H-6'),7.45 (d, *J* = 2 Hz, H-2'), 6.76 (d, *J* = 7.8 Hz, H-5'), 6.26 (d, *J* = 2 Hz,H-8), 6.06 (d, *J* = 2 Hz, H-6), 5.41(d, *J* =7.5 Hz, H-1").

Miquelianin (quercetin 3-O-β-glucuronide) (6)

Yellow powder, PC R_f 0.47 (BAW), 0.46 (15% HOAc). UV λ_{max} (MeOH): 257, 327sh, 359; (NaOMe): 274, 324, 400; ($AlCl_3$): 272, 300sh, 383, 415sh; ($AlCl_3$/HCl): 269, 300, 360, 415sh; (NaOAc): 263, 299, 376; (NaOAc/H_3BO_3): 271, 360. ^{1}H NMR: δ = 12.15 (s, 1H, -OH), 8.4 (d, *J* = 2.2 Hz,H-2'), 7.26 (dd, *J* = 2.2 &8.8 Hz,H-6'), 6.77 (d, *J* = 8.8 Hz, H-5'), 6.31(d, *J* = 2 Hz, H-8), 6.12(d, *J* = 2 Hz, H-6), 5.13(d, *J* = 7.77 Hz, H-1"). ^{13}C NMR: δ = 178.09 (C-4), 172.68 (C-6"), 165.57 (C-9), 161.47 (C-5), 158.35 (C-7), 157.12 (C-2), 148.95 (C-4'), 145.34 (C-3'), 134.69 (C-3), 120.92 (C-1'), 118.88 (C-6'), 115.98 (C-5'), 115.83 (C-2'), 104.05 (C-10), 103.91 (C-1"), 99.59 (C-6), 94.37 (C-8), 77.26 (C-3"), 74.79 (C-5"), 72.42 (C-2"), 72.33 (C-4").

Orientin (luteolin 8-C-β-glucopyranoside) (7)

Yellow powder, PC R_f0.38 (BAW) and 0.18 (15% HOAc). UV λ_{max} (MeOH): 257, 269, 349; (NaOMe): 269, 276, 404; ($AlCl_3$): 272, 304, 426; ($AlCl_3$/HCl): 272, 301, 355, 392; (NaOAc): 270, 356; (NaOAc/H_3BO_3): 265, 379. ^{1}H NMR: δ = 12.8 (s, -OH), 7.54 (dd, *J* = 2& 8.2 Hz, H-6'), 7.53 (d, *J* = 2 Hz, H-2'), 6.86 (d, *J* = 8.2 Hz, H-5'), 6.64(s, H-3), 6.26(s, H-6), 4.69 (d, *J* = 9.64 Hz, H-1").^{13}C NMR: δ = 182.55 (C-4), 164.76 (C-2), 163.58 (C-7),160.79 (C-5), 156.52 (C-9), 150.13 (C-4'), 146.09 (C-3'), 122.18 (C-1'), 120.11 (C-6'), 116.32 (C-5'),113.4 (C-2'), 104.72 (C-8/10),102.82 (C-3), 98.83 (C-6), 81.86 (C-5"), 78.87 (C-2"), 73.72 (C-1"), 71.36 (C-3"), 71.08 (C-4"), 61.94 (C-6")

Isoorientin (luteolin-6-C-β-glucopyranoside) (8)

Yellow powder, PC R_f 0.45 (BAW) and 0.26 (15% HOAc). UV λ_{max} (MeOH): 255, 267, 349; (NaOMe):257, 264, 406; ($AlCl_3$): 272, 304, 426; ($AlCl_3$/HCl): 272, 301, 355, 392; (NaOAc): 271, 395; (NaOAc/H_3BO_3): 265, 379. ^{1}H NMR: δ = 12.8 (s, -OH),7.54 (dd, *J* = 2&8.2 Hz, H-6'), 7.52 (d, *J* = 2 Hz, H-2'), 6.88 (d, *J* = 6.4 Hz, H-5'), 6.65 (s, H-3), 6.48(s, H-8), 4.68 (d, *J* = 8.64 Hz, H-1"). ^{13}C NMR: δ = 182.41 (C-4), 164.71 (C-7), 163.32 (C-2), 160.95 (C-5), 156.52 (C-9), 150.13 (C-4'), 146.09 (C-3'), 122.32 (C-1'), 120.11 (C-6'), 116.32 (C-5'), 112.9 (C-2'), 105.04 (C-6), 102.82 (C-3), 102.84 (C-10), 98.83 (C-8), 81.86 (C-5"), 78.87 (C-2"), 73.72 (C-1"), 71.36 (C-3"), 71.08 (C-4"), 61.94 (C-6").

Apigenin (9)

Yellow powder, PC R_f 0.92 (BAW), 0.15 (15% HOAc). UV λ_{max} (MeOH): 268, 300, 337; (NaOMe): 276, 323, 392; ($AlCl_3$): 276, 302, 345, 384; ($AlCl_3$/HCl): 277, 301, 341, 382; (NaOAc): 269, 304, 383; (NaOAc/H_3BO_3): 269, 304, 357. ^{1}H NMR: δ = 12.91 (s, 1H, -OH), 7.86 (dd, *J* = 2 &8.64 Hz, H-2/6'), 6.80 (dd, *J* = 2 &8.65 Hz,H-3'/5'), 6.67 (s, 1H, H-3), 6.32 (d, *J* = 2 Hz, H-8), 6.04 (d, *J* = 2 Hz, H-6).

Luteolin (10)

Yellow powder, PC R_f 0.85 (BAW), 0.06 (15% HOAc). UV λ_{max} (MeOH): 254, 268, 291sh, 349; (NaOMe): 269, 327sh, 401; ($AlCl_3$): 273, 303sh, 331sh, 421; ($AlCl_3$/HCl): 262, 276, 297sh, 356, 388; (NaOAc): 268, 358; (NaOAc/H_3BO_3): 262, 374. ^{1}H NMR: δ = 12.93 (s, 1H, -OH), 7.35 (dd, *J* = 8.58 Hz, H-6'), 7.33 (d, *J* = 2 Hz, H-2'), 6.82 (d, *J* = 8.48 Hz, H-5'), 6.62 (s, H-3), 6.39 (d, *J* = 2 Hz, H-8), 6.12 (d, *J* = 2 Hz, H-6).

Discussion

In the present study, we focused on the anti-inflammatory and antimicrobial activities of the successive extracts *R. pictus* aerial parts, which were not previously reported. Carrageenan induced paw edema method was used to evaluate the anti-inflammatory activity of the successive extracts of the aerial parts of *R. pictus*. This method is explained by a biphasic event with contribution of different inflammatory mediators. In the first phase (during the first 2 hours after carrageenan injection), histamine and serotonin take the lead, while in the second phase (3–4 hours after carrageenan injection) kinin and prostaglandins are involved (21). The 70% aqueous methanolic extract of *R. pictus* showed the highest anti-inflammatory activity among the tested extracts. This bioactive extract maximally inhibited the edema at the first hour from carrageenan injection; where the inhibition rate reached 68.97% then started to decrease gradually to 57.89% after 4 hours, thus inhibiting various elements and chemical mediators of inflammation. Antimicrobial activity of the successive extracts was assessed using disc diffusion method. Among the tested successive extracts, 70% aqueous methanolic extract significantly inhibited the growth of gram-negative, gram-positive bacteria and yeast. Inhibition of gram-negative bacteria by the extract was the highest, with *S. typhimurium* and *E. coli* being more susceptible (80% and 66.7%, respectively, of ampicillin inhibitory power). Therefore, it was of deemed interest to carry out an in-depth phytochemical study for this bioactive extract. Phytochemical investigation of the 70% aqueous methanolic extract revealed the presence of appreciable percentage of phenolic compounds and flavonoids. Chromatographic analysis led to the isolation and identification of ten compounds, three of which were anthraquinones; emodin (**1**), physcion (**2**) and emodin-8-*O*-*β*-glucoside (**3**), in addition to, seven flavonoids, kaempferol 3-*O*-*β*-glucopyranoside **(4)**,quercetin 3-*O*-*β*-glucopyranoside **(5)**, quercetin 3-*O*-*β*-glucuronide **(6)**, luteolin 8-*C*-*β*-glucopyranoside **(7)**, luteolin 6-*C*-*β*-glucopyranoside **(8)**, apigenin **(9),** and luteolin **(10)**. Compounds **2**-**6** were isolated for the first time from *R. pictus.* According to literature, emodin (**1**), physcion (**2**) and emodin-8-*O*-*β*-glucoside (**3**) have been found to possess laxative, anti-inflammatory, antimicrobial and anticancer activities (22). Besides, flavonoids such as luteolin 8-*C*-*β*-glucopyranoside **(7)**, apigenin **(9),** and luteolin **(10)** have been reported to significantly inhibit inflammation (23,24). Flavonoids are capable of inhibiting exposition of isoforms of inducible nitric oxide synthase, cyclooxygenase, and lipoxygenase, which are in charge of the production of a great amount of nitric oxide, prostanoids, leukotrienes, and other mediators of the inflammatory process (25). Moreover, flavonoids are notorious to be synthesized by plants in response to microbial infection; thus, unsurprisingly they have been found to exhibit remarkable *in vitro* antimicrobial activities against a wide array of microorganisms. Antibacterial flavonoids may have several cellular targets, rather than single site of action (24). Therefore, the isolated compounds have contributed, either individually or in combined form, to the anti-inflammatory and antimicrobial activities of *R. pictus*.

Conclusion

The results of this paper present for the first time, the anti-inflammatory and antimicrobial activities of the 70% aqueous methanolic extract of *R. pictus* Forssk. thus supporting the traditional uses of the plant. However, the probable mechanisms of action and the potential synergetic effects of the isolated compounds need to be further investigated and evaluated. Moreover, further experiments, including *in vivo* and clinical studies, should be implemented to confirm the application of the plants as a medicinally effective plant.

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Authors' contributions

NMA was responsible for selecting the topic and supervising the work. LTAK supervised the extraction and compound isolation. NAA supervised the chemistry part. SHE supervised the chemistry part and revised the manuscript. MEM conducted the antimicrobial part. EMA was responsible for conducting the chemistry part and writing the manuscript. All read and confirmed the final version of the article for publication.

Conflict of interests

All authors declare no conflict of interest associated with this work.

Ethical considerations

All animal handling and procedures were performed according to the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH publication No. 85–23, revised 1996). The current protocol was confirmed by the Medical Ethical Committee of the National Research Centre in Egypt (MEC 1190401) and was in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues.

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