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RESEARCH ARTICLE

PHYTOCHEMISTRY STUDY OF PLANTS BELONGING TO CAPPARIS

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ABSTRACT

Objectives: Bioactive molecules have gained attention in recent time due to their diverse effects and their economic importance. The objective of current study was to isolate, analyze and identify the active constituents from leaves of *C. cartilaginea*.

Methods: The powdered leaves were extracted with different solvents by using Soxhlet apparatus and carried out by different chromatographic techniques. The phytochemical characterizations were evaluated by nuclear magnetic resonance and mass spectrometry.

Results: Three pure compounds were identified representing 1.6% of the total phytochemical composition. Yield of compounds 1, is *Kaempferol* (6gm), compound 2, is Dihydroxy-lup-20(29)-en-28-oic acid (3.8gm) and compound 3, is β -Sitosterol (5gm). Three of them are report for first time from this Spp.

Conclusion: From this study, can be concluded that report for the first time from *C. cartilaginea* leaves, identified 3 pure chemical compounds, by analysis using various physical and spectral techniques.

Keywords: *Capparis cartilaginea* leaves; Kaempferol, 3,23-Dihydroxy-lup-20(29)-en-28-oic acid, β -Sitosterol, NMR analysis.

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INTRODUCTION

Phytochemicals consists of a various group of natural bioactive molecules very distributed in plants. These bioactive molecules have gained attention in recent times due to their diverse effects on human physiology and human health and their economic importance¹. Secondary metabolites are basically classified into three classes namely alkaloids, phenolics and terpenoids². The molecules in these classes are further grouped due to their numerous modifications, thus emphasizing the diversity of secondary metabolites. Considering this, reasonable attention has been focused on the identification of plants rich in bioactive phytochemicals and characterization of their medicinal and economic values³. Nutrient limitation, shunt metabolism produced during idiophase, defense mechanism regulator molecules etc, as a result of secondary metabolism include products an excess metabolism⁴. Medicinal flora can be use as treatment of diseases and therapeutic agents pro management of health because they have power over health enhance effects and have bioactive chemical ingredients³. An earlier investigation has reported that Capparis species extracts, such as *C. spinosa* and *C. decidua* from Saudi

Arabia has a significant anti-inflammatory activity⁵. Newly, the mechanism of anti-inflammatory effect of *C. spinosa* was suggested by El Azhary *et al.*⁵ and involved inhibition of cellular infiltration and cytokine gene expression. Rutin, a flavonoid, obtained from *C. acutifolia* Sweet exhibited a potent anti-inflammatory effect⁶. Other studies have reported the antioxidant, cytotoxic, larvicidal, antimicrobial, hypotensive and bradycardiac activities of *C. cartilaginea*^{3,7,8}. *Capparis cartilaginea* have various traditional uses in the Arab region. It is used for easing bruises, childbirth, earache, headache, paralysis, swelling, skin and joint inflammation, knee problems; tendinitis and snakebites^{5,6}. In Yemen, it is called lattsaf, laşaf or nişaf⁹ and used to treat itching, shortness of breath, head cold, tumors, wounds, boil and for painful knees^{10,11}. Phytochemical studies of *C. cartilaginea* have resulted in isolating flavonoids⁹ and isothiocyanates¹². Other phytochemicals, such as carbohydrates, saponins, polyphenols, flavonoids, tannins, triterpenes, sterols, amino acid and protein have also been found in the leaves of *C. cartilaginea* from Yemen¹³. Capers has medicinal distinctive such as rheumatism. Roots used as diuretic, astringent, and

tonic¹⁴. Bark root used as appetizer, astringent, tonic, antidiarrheic and treat hemorrhoids and spleen disease. Bark was also used for gout and rheumatism, as expectorant, and for chest diseases¹⁵. Infusion of stems and root bark were used as antidiarrheic and febrifuge. Fresh fruits were used in sciatica, and dropsy¹⁶

MATERIALS AND METHODS

All chemicals used in the study were of analytical grade and arranged locally. TLC and preparative TLC were performed using pre-coated aluminium and glass plates with silica gel 60 F254, whereas column chromatography was carried out on silica gels 70-230 mesh. Spots and bands for compounds on TLC were detected using UV light. Proton NMR (500 MHz) and carbon-13 NMR (150 MHz) spectra were recorded on JEOL JNM-ECP400 and chemical shifts in ppm were referenced to internal acetone-d₆ and CDCl₃, respectively.

Figure 1: Kaempferol.

Plant material

Plant collection was done on 26 November 2017 from Al-Mahweet North City in Yemen. Identification and classification of the plant material was performed at the Faculty of Medical Science of the University of Al-Razi specimens were pressed and a voucher specimen (CCJ017) was deposited in a collection housed at the Department of Pharmacy and Pharmacology.

Figure 2: Dihydroxy-lup-20(29)-en-28-oic acid.

Extraction and Isolation

Shade dried leaves of *Capparis cartilaginea* were powdered (7000gm). Extracted by soxhlet apparatus with methanol (x4; 3 days). Collective methanolic under vacuum pressure evaporated to yield 8g of the filtrate, then partition with organic solvent (DCM, EtOAc and MeOH) by adding H₂O to yield with aqueous methanolic (30 g), dichloromethane (9 g) and ethyl acetate (26 g) partition. 26 g of ethyl acetate fraction chromatographed by using silica gel (70-230 mesh, 1500 g, 9x120 cm) C.C., eluting by ethyl acetate and methanol, in order to increase the polarity of solvent and every one of collected partition was 20ml. Jointed fractions and rechromatographed by radial chromatography to give CCI (6.00 mg) known as *Kaempferol* (1), CCII (3.8 mg) known as 3,23-Dihydroxy-lup-20(29)-en-28-oic acid (2) and CCIII (5 mg) known as β -Sitosterol (3).

Figure 3: Sitosterol.

Figure 4: ¹H NMR spectra of Sitosterol.

Kaempferol (1): yellowish powder. ¹H NMR (CD₃COCD₃, 500 MHz): δ 8.10 (1H, d, H-2'and6'), 7.80 (1H, d, H-3'and5'), 6.21 (1H, s, H-8), 5.81 (1H, s, H-6). ¹³C NMR (CD₃COCD₃, 150 MHz): δ 179.8 (C-4), 166.0 (C-7), 163.1 (C-5), 160.8 (C-4'), 158.5 (C-2), 156.9 (C-9), 136.0 (C-3), 133.5 (C-2'), 131.9 (6'), 123.1 (C-1'), 115.9 (C-3'and5'), 106.3 (C-10), 101.0 (C-6), 92.9 (C-8).

Dihydroxy-lup-20-en-28-oic acid (2): white amorphous powder. ¹H NMR (CD₃COCD₃, 500 MHz): δ 4.62, 4.74 (2H, d, H-29), 4.86 (1H, m, H-3), 3.11, 3.45 (2H, s, H-23), 0.79, 0.96, 0.98, 1.03, 1.66 (each 3H, s, Me $\times$ 5). ¹³C NMR (CD₃COCD₃, 150 MHz): δ 179.7 (C-28), 151.0 (C-20), 112.1 (C-29), 76.0 (C-3), 69.1 (C-23), 53.6 (C-5), 52.0 (C-9), 50.3 (C-18), 48.1 (C-19), 44.1 (C-17), 43.2 (C-14), 41.0 (C-

8), 40.0 (C-22), 39.1 (C-13), 38.5 (C-4), 38.0 (C-1), 37.1 (C-10), 37.0 (C-16), 34.6 (C-7), 31.1 (C-21), 28.3 (C-23), 27.4 (C-15), 27.0 (C-12), 25.3 (C-2), 22.1 (C-11), 20.6 (C-30), 19.5 (C-6), 18.1 (C-28), 16.3 (C-25), 16.1 (C-26), 15.5 (C-24), 15.0 (C-27).

Sitosterol (3):- white amorphous powder, ^1H NMR (CD_3COCD_3 , 500 MHz): δ 0.68, 0.80, 0.84, 0.91, 1.06 and 1.11 (H- 18,19,21,26,28,29), 5.41 (1H, t, H-6), 3.93 (1H, m, H-3). ^{13}C NMR (CD_3COCD_3 , 150 MHz): δ 141.5 (C-5), 121.1 (C-6), 77.8 (C-3), 57.1 (C-14), 56.2 (C-17), 52.1 (C-24), 50.3 (C-9), 46.1 (C-25), 42.4 (C-13), 41.0 (C-20), 40.2 (C-12), 37.6(C-4), 37.4 (C-1), 36.6 (C-10), 34.0 (C-22), 32.2 (C-8), 32.0 (C-7), 30.4 (C-23), 29.1 (C-16), 28.4 (C-2), 26.1 (C-28), 25.0 (C-15), 21.4 (C-21), 21.3 (C-11), 20.1 (C-27), 19.5 (C-26), 19.0 (C-19), 13.2 (C-29), 12.0 (C-18).

RESULTS AND DISCUSSION

The present studies explain the phytochemical investigation of the aerial parts of *Capparis cartilaginea* leaves which resulted in the isolation and structure elucidation of three compounds, hitherto unreported from this part of plant. The structures of these compounds were elucidated through superior spectroscopic techniques.

Compound (1); yellowish powder isolated. IR scale found hydroxyl group at (3395 cm^{-1}) and carbonyl

group at (1707 cm^{-1}). The molecular formula is ($\text{C}_{16}\text{H}_{20}\text{O}_5$). The chemical shifts and coupling constants of ^1H -NMR data indicated a dihydroxylated pattern for ring A (two *m*-coupled doublet at δ H 6.21 (1H, s, H-8) and 5.81 (1H, s, H-6) and 4'-2'and6'), 7.80 (2H, d, H-3'and5'). Fifteen carbon signal come out in ^{13}C NMR data as well as six methins and nine quaternary carbons at δ 158.5 and136.0, alkenes carbons group appeared.

Compound (2); purification as white amorphous powder. ($\text{C}_{30}\text{H}_{50}\text{O}_4$) is the molecular formula for the compound. Proton ^1H -NMR data exhibit the presence of five tertiary methyl groups at δ H 0.79, 0.96, 0.98, 1.03 and 1.66 for H-(24, 25, 26, 27, 30) positions respectively. The ^{13}C -NMR spectrum of compound display carbonyl of acidic acid, five tertiary methyl groups, terminal double bond and twenty three signals to other carbon established in ^{13}C -NMR data.

Compound (3); was purified as white amorphous powder. ^1H NMR data appeared at δ (0.68, 0.80, 0.84, 0.91, 1.06 and 1.11), for six methyl groups. Multiple (H-3), Proton appeared at δ (3.93). Olefinic protons present at δ 5.41. Spectrum of ^{13}C NMR showed twenty nine different carbon groups as signal for six methyl's, nine methylene's, eleven methins and three quaternary carbons. Also appeared at δ 141.5 and 121.1, for alkenes carbons.

Figure 5: ^{13}C spectra for sitosterol.

Figure 6: ^1H NMR spectra of Kaempferol.

Figure 7: ^{13}C NMR spectra of Kaempferol.

CONCLUSION

Kaempferol, dihydroxy-lup-20(29)-en-28-oic acid and Sitosterol isolation and purification from the leaves of *Capparis cartilaginea*. The identification and reporting was done for the first time from this part of the plant. The isolation, purification and analysis carried out by means of various physical (solvent extraction, column chromatography, radial chromatography, preparative TLC and melting points) and spectral techniques.

AUTHOR'S CONTRIBUTION

The manuscript was carried out, written, and approved in collaboration with all authors.

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CONFLICT OF INTEREST

No conflict of interest associated with this work.

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