Phytochemical Isolation and Biological Activities of *Corydalis adiantifolia* from Baltistan

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Summary: The whole plant material of *Corydalis adiantifolia* Hook.f. & Thomson was investigated for biological activities e.g. antifungal, antibacterial, insecticidal, cytotoxic, anticancer and phytotoxic activities and preliminary phytochemical screening. The methanol extract and dichloromethane fraction of the plant species exhibited 5% inhibition each against the fungus *Aspergillus flavus*. The methanol extract and water and dichloromethane fractions exhibited non-significant antibacterial activity and they showed inhibition against Hela cell lines and insignificant insecticidal activity. To understand the bioactive profile of *C. adiantifolia*, phytochemical screening approach is considered effective. The samples including methanol extract and *n*-hexane, dichloromethane, ethyl acetate and water fractions were subjected to qualitative phytochemical screening for the presence of various phytochemicals i.e. alkaloids, flavonoids, saponins, diterprenes, triterpenoids, anthraquinones, anthranol glycosides, reducing sugars and phenols. The results exhibited the efficacy of methanol extract showing the presence of more phytochemicals in comparison to the fractions of *C. adiantifolia*. Moreover, as a result of phytochemical isolation, 8-acetonyldihydrosanguinarine (**1**), β-sitosterol (**2**) and β-sitosterol-β-D-glucoside (**3**) were isolated, purified, and characterized by spectroscopic methods. To the best of our knowledge, all this study was carried out for the first time on *C. adiantifolia*.

Key Words: *Corydalis adiantifolia*, Herbal hair tonic, Phytochemistry, Biological activity, Baltistan.

Introduction

Corydalis adiantifolia Hook.f. & Thomson (Fumariaceae) is a herb locally named as shampoo (Balti) and reported as hair tonic in Shigar valley Baltistan [1] and also this plant is used for the treatment of eye diseases or eye-sight improvement in Chapursan valley Gilgit [2]. The family Fumariaceae is, in Pakistan, represented by 30 plant species distributed in two genera viz., *Corydalis* Medik and *Fumaria* L. [3]. *Corydalis* species viz., *C. falconeri* and *C. stewartii* are generally known as Shorot (Balti) in Baltistan region, and reported for the treatment of various eye diseases, fever, cough and also as hair tonic [4]. In addition certain *Corydalis* species have been used as antidiabetic [5], anticancer [6, 7], anti-inflammatory [8, 9], antinoceptive [10], hepatoprotective [11], and analgesic [12] etc. Phytochemical study showed that mostly alkaloids [13-15] have been reported from various *Corydalis* species.

The present study was aimed to evaluate the methanolic extract of *C. adiantifolia* for miscellaneous biological activities including antifungal, antibacterial, insecticidal, cytotoxic, anticancer and phytotoxic. Moreover, *C. adiantifolia* was studied for preliminary phytochemical screening and isolation of the phytoconstituents. Because of our study, 8-acetonyldihydrosanguinarine (**1**), β-sitosterol (**2**) and β-sitosterol-β-D-glucoside (**3**) were isolated and characterized for the first time from *C. adiantifolia*.

Experimental

Plant material: The whole plant material of *C. adiantifolia* (Fumariaceae) was collected from Shigar, Baltistan in August 2015 and was authenticated by Dr. Sher Wali Khan, Department of Biological Sciences, Karakoram International University (KIU), Gilgit, Pakistan. The voucher specimen (SKN-01) was deposited to the Department of Biological Sciences, KIU. Sample was washed with tap water to remove the dust, soil particles and other pollutants. Whole plant material was collected, dried in shade and crushed to powder using a grinder.

Extraction and fractionation: The crushed form of *C. adiantifolia* (1kg) was soaked and extracted with 100% MeOH (3L) for a week at room temperature. The methanol extract (CAM) was

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evaporated to dryness using rotary evaporator at 40°C under reduced pressure. CAM (25g) was further fractionated with *n*-hexane (CAH), dichloromethane (CAD), and ethylacetate (CAE) and water (CAW) residue was left behind.

Preliminary phytochemistry screening: Preliminary qualitative phytochemical investigation was carried out for all the extract and fractions as per the standard methods [16-19]. In brief, the protocols are discussed below.

Test for alkaloids

Mayer's test: The test samples were treated with Mayer's reagent. Yellow precipitates formation was an indication of alkaloids.

Wagner's test: The test samples were treated with Wagner's reagent, formation of brown/reddish precipitates indicated the presence of alkaloids.

Dragendorff's test: Samples were spotted on TLC plate and Dragendorff's reagent was applied. Alkaloids were confirmed by the formation of red precipitates.

Hager's test: Samples were treated with Hager's reagent. Alkaloids were confirmed by the formation of yellow precipitates.

Test for flavonoids

Alkaline reagent test: The samples were treated with NaOH solution. Formation of intense yellow color, which becomes colorless on addition of dilute acid, indicated the presence of flavonoids.

Lead acetate test: A small amount of extract was taken in a test tube and few drops of lead acetate solution were added. Yellow colored precipitates were formed which indicated the presence of flavonoids.

H_2SO_4 test: Few drops of H_2SO_4 were added to the extract in a test tube. An orange color showed the presence of flavonoids.

Test for saponins

Froth test: The test samples were diluted with distilled water to 20ml. Then 15ml solution was taken in graduated cylinder and shaken well for 15 minutes. The formation of 1cm foam layer on the surface confirmed the presence of saponins.

Foam test: 2ml water was added to 0.5g of sample and shaken it for a few minutes. Foam persisted on the top for 10 minutes and it was an indication of saponins.

Test for terpenoids

Salkowski's test: The plant extract (2.5mg) is dissolved in chloroform (1ml) and concentrated H_2SO_4 (1.5ml) was added carefully to form a layer. In inner face, a reddish brown color appeared that was indication for terpenoids.

Test for diterpenes: A small quantity of extract was dissolved in 3ml distilled water. Then 3-4 drops of 3% copper acetate solution were added. Emerald green color appeared which indicated the presence of diterpenes.

Test for triterpenoids: The crude extract (5mg) was dissolved in chloroform (2ml) and acetic anhydride (1ml) was added to the solution. On addition of concentrated H_2SO_4 (1ml) to the solution, a reddish violet color appeared that indicated the presence of triterpenoids.

Test for anthraquinones

Borntrager's test: The test extract (5mg) was dissolved in 3ml of 10% HCl and the mixture was boiled in water bath for few minutes. It was filtered and allowed to cool. Then 3ml of $CHCl_3$ was added to the filtrate. When it was treated with few drops of 10% NH_3 pink color was formed that indicated the presence of anthraquinones.

Test for anthranol glycosides

Modified Borntrager's test: The samples were treated with ferric chloride solution and immersed in boiling water for about 5 minutes. The mixture was cooled and extracted with equal volumes of benzene. The benzene layer was separated and treated with ammonia solution. Formation of rose-pink color in the ammonical layer indicated the presence of anthranol glycosides.

Test for reducing sugars

Benedict's test: The samples were treated with Benedict's solution and heated. Appearance of orange red precipitates indicated the presence of reducing sugars.

Fehling's test: The test samples were hydrolyzed with hydrochloric acid and treated with a base (neutralized). Both Fehling solution A and Fehling solution B were added and heated. Reducing

sugars were confirmed by the formation of red precipitates.

Test for phenols:

Ferric chloride test: Few drops of $FeCl_3$ were added to test extract (10mg). Bluish black color appeared which indicated the presence of phenols.

Chromatography of methanolic extract: The methanolic extract of *C. adiantifolia* was chromatographed with different solvents starting from *n*-hexane, EtOAc and MeOH. As a result, F1-F77 fractions were obtained from the main column elution. The major fraction F29 obtained from the main column with elution of 50% EtOAc:*n*-hexane was re-chromatographed over normal silica gel and the sub-fractions CA-29-1 to CA-29-26 were obtained. CA-29-11 yielded compound **1**, CA-29-26 yielded compound **2**. And the major fraction F-53 (2% MeOH:EtOAc) yielded compound **3**. The structures of compounds **1-3** were confirmed by comparing their NMR data with those reported in literature.

Antibacterial activity (96 well plate method): Organisms were grown in Mueller Hinton medium, inoculums were adjusted to 0.5 McFarland turbidity index. Stock solutions of each plan extract were prepared in DMSO (1:1). Media was dispensed to all wells and the work was triplicated. The test sample was added in wells, control wells were kept without any test. The volume of each well plate was made up to 200µl and finally 5x106 cells in all wells including both control and test were added. The plates were sealed using parafilm and incubated for 18-20hrs. Alamar Blue Dye was dispensed in each well, covered with foil and shaken at 80 RPM in a shaking incubator for 2-3hrs. The growth in bacterial strains was indicated by change in color of Alamar Blue dye from blue to pink. Absorbance was recorded at 570 and 600nm by the ELISA reader [20, 21].

Antifungal activity (agar tube dilution method): 24mg of crude extract and 12mg of pure compound were dissolved in 1ml sterile DMSO serving as stock solution. Sabouraud dextrose agar (SDA) was used for the growth of fungus. Media (pH 5.5-5.6) with high concentration of well dissolved glucose/maltose (2%) was prepared (32.5g per 500ml distilled H_2O), and dispensed 4ml into screw caps tubes and autoclaved at 121°C for 15 min. Tubes were allowed to cool (50°C) and non-solidified SDA was loaded with 66.6µl of compound from the stock solution. The final concentrations of 400µg/ml (crude extract) and 200µg/ml (media for pure compound) were prepared. Tubes were solidified in slanting position at room temperature. Each tube was inoculated with 4mm fungus piece (seven-day-old fungus culture). For non-mycelial growth, an agar surface streak was used. Other media supplemented with DMSO and reference antifungal drug were used as negative and positive control respectively. The tubes were incubated at 27-29°C for 3-7 days. Cultures were analyzed twice in a week during incubation. Growth in the compound amended media was determined by measuring linear growth (mm) and growth inhibition was calculated with reference to the negative control [22, 23]. The percent inhibition of fungal growth is calculated by the given equation:

$$\% \text{ inhibition} = \frac{\text{100- linear growth in test (mm)}}{\text{linear growth in control (mm)}} \times 100$$

Anticancer activity (Hela cell lines): Cytotoxicity was evaluated in 96-well flat-bottomed micro plates using the standard MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) colorimetric assay. Hela cells were cultured in minimum essential medium eagle, supplemented with 5% of fetal bovine serum (FBS), 100IU/ml of penicillin and 100µg/ml of streptomycin in 75cm^2 flasks, and kept in 5% CO_2 incubator at 37°C. Exponentially growing cells were harvested, counted with haemocytometer and diluted with a particular medium. Cell culture ($6x10^4$ cells/ml) was prepared and introduced (100µl/well) into 96-well plates. After overnight incubation, medium was removed and 200µl of fresh medium with sample (1-30µM) was added. After 48hrs, 200µL MTT (0.5mg/ml) was added to each well and incubated (4hrs). Subsequently, 100µL DMSO was added to each well. The extent of MTT reduction within cells was calculated by measuring the absorbance at 570nm, using a microplate reader (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as IC_{50} for HeLa [24]. The percent inhibition was calculated by using the following formula and the percent inhibitions were processed using Soft- Max Pro software (Molecular Device, USA).

$$\% \text{ inhibition} = \frac{\text{100-(mean of O.D of test -mean of O.D of negative control)}}{\text{(mean of O.D of positive control-mean of O.D of negative control)}} \times 100$$

Cytotoxic activity (3T3 cell lines): A standard MTT colorimetric assay was followed and the samples were evaluated in 96-well flat-bottomed micro plates. 3T3 cells were cultured in Dulbecco's modified eagle medium, supplemented with 5% of

FBS, 100IU/ml of penicillin and 100µg/ml of streptomycin in 75cm^2 flasks, and kept in 5% CO_2 incubator at 37ºC. Exponentially growing cells were harvested, counted and diluted using particular medium. Cell culture (5x10^{4}cells/ml) was prepared and introduced (100µL/well) into 96-well plates. After overnight incubation, medium was removed and 200µL of fresh medium containing sample (1-30µM) was introduced. After 48hrs, 200µl MTT (0.5mg/ml) was added to each well and incubated (4hrs). Subsequently, 100µL of DMSO was added to each well. The extent of MTT reduction was calculated using a micro plate reader by measuring the absorbance at 540nm (Spectra Max plus, Molecular Devices, CA, USA). The cytotoxicity was recorded as IC_{50} for 3T3 cells [24]. The percent inhibition was calculated by using the following formula and the results were processed using Soft-Max Pro software (Molecular Device, USA).

$$\% \text{ inhibition} = \frac{100-(\text{mean O.D of test -mean O.D of neg. control})}{(\text{mean O.D of pos. control-mean O.D of neg. control})} \times 100$$

Phytotoxic activity: E-medium was prepared in one liter dist. H_2O and pH was adjusted between 6.0 to 7.0 by adding KOH pellets (stock solution). Working E-medium was prepared by mixing 100ml of stock solution and 900ml of dist. H_2O. 30mg of study sample was dissolved in 1.5ml of solvent (MeOH/EtOH etc.) serving as stock solution. Three flasks were inoculated with 10, 100 and 1000µl of solution pipetted from the stock solution and were left overnight to evaporate the solvent. 20ml (E. medium) was added and then *Lemna minor*, containing a rosette of 2-3 fronds, was added to each flask (total 20 fronds). Other flasks were supplemented with E-medium and reference (standard drug) plant growth inhibitors and promoters served as -ve and +ve controls, respectively. The flaks were placed in growth cabinet for seven days. Plants were examined daily during incubation. The number of fronds were counted and recorded per flask on day 7. Results were analyzed as growth regulation in percentage, calculated with reference to the -ve control using the given formula [25, 26].

$$\% \text{ regulation} = \frac{100-\text{no. of fronds in test sample}}{\text{no. of fronds in negative control}} \times 100$$

Insecticidal activity (impregnated filter paper method): The study sample was prepared by adding 3ml solvent (EtOH, MeOH, Acetone etc.) in 200mg test sample. The stored grain pests were reared in the laboratory under controlled conditions (temperature and humidity) in plastic bottles containing sterile breeding media. The test insects (*Tribolium castaneum, Sitophilus oryzea, Rhyzopertha dominica, Trogoderma granarium and Callosobruchus analis*) of uniform age and size were used for the experiment. In the first day, the filter paper was cut according to the size of petri plate (9cm or 90mm) and put them in the plate. The whole sample was loaded over the filter paper with the help of micropipette. The plates were left for 24 hours to evaporate the solvent completely. In the subsequent day, 10 insects of each species were put in each plate (test and control) with the help of a clean brush. Healthy and active insects of same size and age were taken. The plates were incubated at 27°C for 24 hours with 50% relative humidity in growth chamber. In the third day, the survival of the insects (count the number of survivals of each species) was assessed. The % inhibition or % mortality was calculated with the help of the following equation:

$$\% \text{ mortality} = \frac{100-\text{no. of insects test sample}}{\text{no. of insects alive in control}} \times 100$$

The positive (standard insecticide, Permethrin) and negative (volatile solvent and test insect) controls were run with test sample [27].

Results and Discussion

Phytochemical investigation of *C. adiantifolia*: The methanol extract (CAM), *n*-hexane (CAH), dichloromethane (CAD), ethyl acetate (CAE) and water (CAW) fractions were studied qualititavely for the presence of alkaloids, flavonoids, saponins, terpenoids, diterpenes, triterpenoids, anthraquinones, anthranol glycosides, reducing sugars and phenols using different test protocols. The methanol (CAM) sample was found to possess all types of the phytoconstituents. The other samples i.e. CAH, CAD, CAE and CAW showed positive results for alkaloids using Mayer's and Wagner's test protocols and triterpenoids using Salkowski test protocol. Furthermore CAH and CAD showed negative results for flavonoids and phenols. Diterpenes, flavonoids (using lead acetate test), and anthranol glycosides were found absent in CAD and CAW. While CAH, CAD, CAE and CAW were not tested for alkaloids using Hager's test and Dragendorff's test protocols. The miscellaneous results of preliminary phytochemical screening of *C. adiantifolia* are described in Table-1.

Table-1: Preliminary phytochemical screening of extract/fractions of *C.adiantifolia.*

S#	Phytochemical constituents	Test Protocol	CAM	CAH	CAD	CAE	CAW
1	Alkaloids	Mayer's test	+ve	+ve	+ve	+ve	+ve
		Wagner's test	+ve	+ve	+ve	+ve	+ve
		Hager's test	+ve	NT	NT	NT	NT
		Dragendorff's reagent test	+ve	NT	NT	NT	NT
2	Flavonoids	Alkaline reagent test	+ve	-ve	-ve	+ve	+ve
		Lead acetate test	+ve	-ve	-ve	+ve	-ve
		H_2SO_4 test	+ve	-ve	-ve	+ve	+ve
3	Saponins	Froth test	+ve	+ve	+ve	+ve	+ve
		Foam test	+ve	NT	NT	NT	NT
4	Terpenoids		+ve	+ve	+ve	NT	NT
5	Diterpenes		+ve	+ve	-ve	+ve	-ve
6	Triterpenoids	Salkowski test	+ve	+ve	+ve	+ve	+ve
7	Anthraquinones	Borntrager's test	+ve	NT	+ve	NT	NT
8	Anthranol glycosides	Modified Borntrager's test	+ve	-ve	+ve	-ve	-ve
9	Reducing sugars	Benedict's test	+ve	NT	NT	NT	+ve
		Fehling test	+ve	NT	NT	NT	+ve
10	Phenols		+ve	-ve	-ve	+ve	+ve

*CAM = *C. adiantifolia* methanolic extract; CAH = *C. adiantifolia n*-hexane extract; CAD = *C. adiantifolia* dichloromethane extract; CAE = *C. adiantifolia* ethyl acetate extract; CAW = *C. adiantifolia* water extract; NT = Not tested

Furthermore, phytochemical investigation of *C. adiantifolia* resulted in the isolation of three compounds namely 8-acetonyldihydrosanguinarine (**1**), β-sitosterol (**2**) and β-sitosterol-D-glucoside- (**3**) and their structures were determined by means of spectral analysis and compared with available literature (Figure 1).

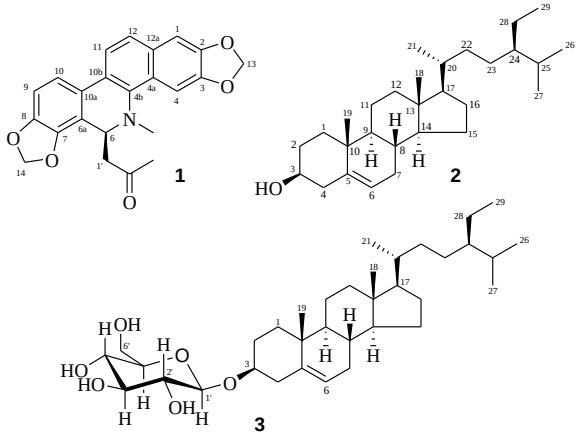

Fig. 1: Structures of compounds **1-3** isolated from *C. adiantifolia.*

8-Acetonyldihydrosanguinarine (**1**)

^{1}H-NMR (DMSO, 400 MHz) (δ ppm): 7.78 (1H, d, *J* = 7.8Hz), 7.56 (1H, d, *J* = 7.6Hz), 7.44 (1H, d, *J* = 7.4Hz), 7.33(1H, s), 7.30 (1H, s), 6.95 (1H, d, *J* = 7.0Hz), 4.75 (1H, dd, *J* = 4.6, 14.7Hz), 2.53 (3H, s, NCH_3), 2.06 (3H, s, CH_2COCH_3), 6.10-6.14 (4H, m, 2-OCH_2O), 2.22-2.39 (2H, m, CH_2COCH_3). ^{13}C-NMR ($CDCl_3$, 100 MHz) (δ ppm): 30.9 (CH_2COCH_3), 42.7 (N-Me), 46.2 (CH_2COCH_3), 56.5 (C-6), 99.7 (C-4), 101.8 (2,3-OCH_2O-), 101.9 (7,8-OCH_2O-), 104.6 (C-1), 109.3 (C-9), 116.0 (C-10b), 119.9 (C-10), 120.3 (C-11), 123.3 (C-6a), 124.0 (C-12), 124.3 (C-10a), 128.8 (C-4a), 131.5 (C-12a), 139.2 (C-4b), 145.9 (C-7), 147.2 (C-8), 147.5 (C-3), 148.8 (C-2), 207.4 (CH_2COCH_3). MS (EI +ve ion mode) *m/z*: 389 (27), 333 (56), 332 (100), 317, 274, 166, 143, 75, 43. The spectral data was compared with previous literature [28-30] which supported that compound (**1**) is 8-acetonyldihyrosanguinarine.

β-Sitosterol (**2**):

^{1}H-NM R ($CDCl_3$, 300 MHz) (δ ppm): 5.27-5.29 (1H, m), 3.39-3.51 (1H, m), 2.10-2.27 (2H, m), 1.85-1.98 (2H, m), 1.70-1.82 (3H, m), 1.35-1.64 (10H, m), 0.99-1.31 (13H, m), 0.94 (3H, s), 0.85 (3H, d, *J* = 6.48 Hz), 0.73-0.80 (9H, 3Me, m), 0.61 (3H, s). ^{13}C-NMR ($CDCl_3$, 500 MHz) (δ ppm): 140.76 (C-5),121.71 (C-6), 71.81 (C-3), 56.76 (C-14), 56.05 (C-17), 50.13 (C-9), 29.15 (C-25), 42.30 (C-13), 42.30 (C-4), 39.77 (C-12), 37.25 (C-1), 36.50 (C-10), 33.94 (C-20), 45.84 (C-22), 31.67 (C-7), 31.67 (C-8), 31.90 (C-2), 12.04 (C-24), 28.24 (C-16), 24.29 (C-23), 26.07 (C-15), 19.39 (C-28), 21.07 (C-11), 18.81 (C-27), 23.06 (C-26), 19.03 (C-19), 26.07 (C-21), 11.9 (C-29), 36.14 (C-18). MS *m/z*: 414, 381, 303, 145, 43. The spectral data was compared with previous literature [31] which supported that compound (**2**) is β-sitosterol.

β-Sitosterol-3-O-β-D-glucoside (**3**)

^{1}H-NM R ($CDCl_3$, 300 MHz) (δ ppm): 5.15 (1H, m, H-6), 4.18 (1H, d, *J* = 7.76 Hz, anomeric H of glucose), 3.64 (1H, t, J = 3.15 Hz), 3.55 (1H, d, *J* = 4.58 Hz), 3.52 (1H, d, J = 4.56 Hz), 3.32-3.40 (1H, m), 3.21-3.25 (2H, m), 3.14-3.16 (2H, m), 3.00-3.08 (2H, m), 2.19-2.20 (1H, m), 2.02-2.08 (1H, m), 1.63-1.81 (5H, m), 1.14-1.47 (8H, m), 0.85-1.13 (12H, m), 0.79 (3H, s), 0.67-0.73 (5H, m), 0.55-0.65 (10H, m), 0.47 (3H, s); ^{13}C-NMR ($CDCl_3$, 500 MHz) (δ ppm):

140.18 (C, C-5), 122.03 (C-6), 76.68 (C-3), 56.58 (C-14), 55.88 (C-17), 50.00 (C-9), 45.68 (C-24), 42.15 (C-13), 39.58 (C-4), 38.53 (C-12), 37.07 (C-1), 36.54 (C-10), 35.97 (C-20), 33.77 (C-22), 31.75 (C-7), 31.70 (C-8), 29.44 (C-2), 28.97 (C-25), 28.06 (C-16), 25.88 (C-23), 24.11 (C-15), 22.88 (C-28), 20.88 (C-11), 19.59 (C-26), 19.12 (C-19), 18.80 (C-27), 18.57 (C-21), 11.75 (C-29), 11.65 (C-18), chimerical shift values observed for sugar carbons are, 100.89 (C-1′), 77.00 (C-3′), 76.19 (C-5′), 73.35 (C-2′), 69.98 (C-4′), 61.72 (C-6′). MS *m/z*: 576 [M^+] (for $C_{35}H_{60}O_6$), 414 [M^+ - glucose], 381, 329, 303, 273, 255, 213, 159, 145, 119, 107, 81, 55, 43. The spectral data was compared with previous literature [32] which supported that compound (**3**) is β-sitosterol-3-O-β-D-glucoside.

Biological activities: The antifungal activity of different extracts of *C. adiantifolia* was carried out against fungi *viz. Trichophylon longifusus, Candida albicans, Aspergillus flavus, Microsporum canis, Candida glabirata* and *Fusarium solani.* Miconazole and amphotericin B were the standard drugs used in the tests. The samples CAM, CAD and CAW showed no significant activity against above fungi, but CAM and CAD exhibited 5% inhibition each against *A. flavus* with MIC (µg/ml) 20.70. The results are provided in Table-2.

On the other hand, the antibacterial activity of samples was tested against bacteria *viz. Escherichia coli, Bacillis subtilis, Staphylococcus aureus, Pseudomonas aeruginosa and Salmonella typhi.* The stock solution for each sample was prepared with concentration 3000 µg/ml. The maximum % inhibition exhibited by water extract (CAW) was found 26% against *E. coli* followed by 8.19% against *B. subtilis* and 7.86% against *P. aeruginosa*. The lowest % inhibition was observed by the water extract against *S. typhi* which was only 4.5%. Similarly the maximum % inhibition (i.e. 19.95%) by the methanol extract (CAM) was observed against *E. coli* and the lowest % inhibition by CAM was exhibited against *S. typhi*. In case of the dichloromethane extract (CAD), the sample showed 22.32%, 21.99%, and 14.87% inhibition against *E. coli*, *B. subtilis*, and *S. aureus* respectively. While the maximum % inhibition was observed against *P. aeruginosa* and the lowest % inhibition was seen against *S. typhi*. The samples exhibited non-significant activity and the results are given in Table-3.

Moreover, the cytotoxicity (3T3 cell line) activity was carried out and cyclohexamide was used as standard against normal cell line. The study samples CAW, CAM and CAD (30µg/ml each) exhibited 20, 33 and 38% inhibition respectively as compared with the standard (70%) in concentration similar to the study samples. The results are given in Table-4.

Table-2: Results of *in vitro* antifungal activity.

Name of Fungus	Sample mm	Control mm	% inhibition			Std. Drug	MIC (µg/ml)
			CAW	CAM	CAD		
Candida albicans	**100**	**100**	**0%**	**0%**	**0%**	**Mic**	**97.8**
Trichphyton longifusus	**100**	**100**	**0%**	**0%**	**0%**	**Mic**	**113.5**
Aspergillus flavus	**100**	**100**	**0%**	**5%**	**5%**	**Amp**	**20.70**
Microsporum canis	**100**	**100**	**0%**	**0%**	**0%**	**Mic**	**98.1**
Fusarium lini	**100**	**100**	**0%**	**0%**	**0%**	**Mic**	**73.50**

*CAW = *C. adiantifolia* water extract; CAM = *C. adiantifolia* methanolic extract; CAD = *C. adiantifolia* dichloromethane extract

Table-3: Results of antibacterial activity.

Name of Bacteria	%inhibition (3000 µg/ml)			
	CAW	CAM	CAD	Ofloxacin
Escherichia coli	**26%**	**19.95%**	**22.32%**	**95.52%**
Bacillus subtilis	**8.19%**	**13.53%**	**21.99%**	**95.19%**
Staphylococcus aureus	**7.63%**	**10.53%**	**14.87%**	**90.93%**
Pseudomonas aeruginosa	**7.86%**	**14.47%**	**30.54%**	**90.99%**
Salmonella typhi	**4.5%**	**10.36%**	**11.70%**	**92.14%**

*CAW = *C. adiantifolia* water extract; CAM = *C. adiantifolia* methanolic extract; CAD = *C. adiantifolia* dichloromethane extract

Table-4: Results of cytotoxicity activity.

Sample Codes	Concentration of Samples (µg/ml)	% inhibition
CAW	**30**	**20%**
CAM	**30**	**33%**
CAD	**30**	**38%**
Cyclohexamide	**30**	**70%**

*CAW = *C. adiantifolia* water extract; CAM = *C. adiantifolia* methanolic extract; CAD = *C. adiantifolia* dichloromethane extract

The anticancer activity was determined against Hela cell lines and the samples *i.e.* CAW, CAM and CAD showed 21, 36 and 28% inhibition respectively as shown in Table-5.

Table-5: Results of anticancer activity.

Sample Code	Concentration (µg/ml)	% inhibition
CAW	30	21%
CAM	30	36%
CAD	30	28%
Cyclohexamide	30	70%

*CAW = *C. adiantifolia* water extract; CAM = *C. adiantifolia* methanolic extract; CAD = *C. adiantifolia* dichloromethane extract

Furthermore, the *in-vitro* phototoxic activity was performed for CAM, CAW and CAD extracts with various concentrations *i.e.* 10, 100 and 1000 (µg/ml). The samples with concentration 10µg/ml showed no inhibition. With 100µg/ml concentration, CAM, CAW and CAD exhibited 29.6, 0 and 12.5% growth inhibition respectively. While with 1000µg/ml concentration CAM, CAW and CAD exhibited 59.37, 95 and 70.8% growth inhibition respectively as represented in Table-6.

Table-6: Results of phytotoxic activity.

Sample code	Concentration (µg/ml)	%Inhibition
	10	0%
CAM	100	29.6%
	1000	59.37%
	10	0%
CAW	100	0%
	1000	95%
	10	0%
CAD	100	12.5%
	1000	70.8%

The study samples CAM, CAW and CAD were also studied for insecticidal activity and all samples exhibited insignificant activity against *Sitophilus oryzae* and *Rhyzopertha dominica* as shown in Table-7.

Table-7: Results of insecticidal activity.

Name of Insects	%Mortality (239.5 µg/cm^2)		Samples (1019.10 µg/cm^2)		
	+ve control	-ve control	CAM	CAW	CAD
Sitophilus oryzae	100%	0%	0%	0%	0%
Rhyzopertha dominica.	100	0%	0%	0%	0%

Recently alkaloids have been reported from certain *Corydalis* species [33, 34] and flavonoids have been reported from *C. bungeana* [35]. On the other hand anthraquinones, terpenes and steroids have also been reported from *C. yanhusuo* [36]. Our present investigation also suggests the presence of alkaloids, flavonoids, terpenes and anthraquinones. As a result of our present study, 8-acetonyldihydrosanguinarine (syn: 6-acetonylsanguinarine. 6-acetonyldihydrosanguinarine); an alkaloid was isolated and characterized. This compound has also been reported from various *Corydalis* species [28, 29] and some other plant species [37-39]. Biological study demonstrated that 8-acetonyldihydrosanguinarine has been reported inactive against *S. aureus*, *E. coli*, *A. hydrophila* and *P. multocida* [30].

Conclusions

The preliminary phytochemical investigation on *C. adiantifolia* reveals the presence of alkaloids, flavonoids, anthraquinones etc. As a result of our present study, 8-acetonyldihydrosanguinarine (**1**), β-sitosterol (**2**) and β-sitosterol-D-glucoside- (**3**) are being reported for the first time. The methanolic extract of the plant was also investigated for difference biological activities, and the samples exhibited non-significant antibacterial activity and good *in-vitro* phytotoxic activity with increased concentration.

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Conflict of interest

The authors declare no conflict of interest.

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