

EFFECT OF SOLVENT CONCENTRATIONS ON QUANTITATIVE ESTIMATION OF TOTAL PHENOLIC AND FLAVONOID CONTENTS OF AERIAL PARTS OF *CALOTROPIS PROCERA* (Ait.) R.Br.

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Phenols and flavonoids present in medicinal plants are considered to be among the most important bioactive components. *Calotropis procera* (Ait.) R.Br. (Family, Apocynaceae) is a medicinal plant which showed a wide range of bioactivity. The aim of this study was to evaluate the effect of solvent concentration on total phenolic content (TPC) and total flavonoid content (TFC) in different extracts of leaves, stems and fruits of *C. procera*. TPC and TFC were estimated by Follin Ciocalteu colorimetric assay and aluminum chloride method using tannic acid and quercetin as standards, respectively. This study showed significant variation in TFC and TPC levels in between the different samples. *C. procera* presented highest both TPC and TFC in leaves extracted with 80% aqueous MeOH. In conclusion, the present investigation demonstrates that there is significant effect of solvent concentration used for extraction of aerial parts of *C. procera* on their TPC and TFC contents.

Keywords: *Calotropis procera*, total phenolic, total flavonoid, tannic acid, quercetin.

INTRODUCTION

Herbal secondary metabolites are prolific sources for pharmaceuticals and are widely used traditionally because of their low side effects. Phenolic compounds and flavonoids are considered the most important bioactive components present in herbal plants. These compounds are characterized by the presence of several phenol groups. They have the ability to donate a hydrogen atom or an electron, which makes them very reactive in chelating free radicals and to be considered as natural antioxidants. In addition, they were proved to have a wide range of biological activities¹⁻³. Recently, several flavonoids and phenolic compounds showed antiviral activities against SARS-CoV2, the virus which causes COVID-

19 outbreak⁴⁻⁸. *Calotropis procera* (Ait.) R.Br., is a medicinal plant with a wide range of biological activities. It is a woody, evergreen, and perennial shrub belongs to the milkweed family Apocynaceae (Asclepiadaceae) which exuding latex from all parts of the plant. It is known worldwide by different common names as the sodom apple, dead sea apple and swallow wort. In Arabic, it is named (usher or debaj). The plant is native to West, North and East Africa, Saudi Arabia, United Arab Emirates, Pakistan, India, Nepal, Afghanistan and South Asia. It grows in different desert areas with few rainfall and sandy soil in Egypt such as Sinai, New Valley, Cairo-Ismailia, and Cairo-Suez way⁹⁻¹¹. *C. procera* contains biologically active constituents including cardenolides, tannins, steroids, phenols,

flavonoids, saponins, and alkaloids. It is used in folk medicine to treat leprosy, asthma, catarrh, and as a rubefacient, an emetic, and a purgative⁹. The plant has shown a wide range of pharmacological activities such as anticancer, antifungal, hepatoprotective, anti-inflammatory, antimicrobial, antipyretic, analgesic, and wound healing activities. Additionally, the plant has antifertility, anthelmintic, antihyperglycemic, anticonvulsant, and antimalarial activities¹². As well as, different extracts of *C. procera* showed antiviral activity against foot-and-mouth disease virus (FMDV), white spot syndrome virus (WSSV), human immunodeficiency virus (HIV-1) and the plant virus, Tobacco mosaic virus (TMV)¹³. Several phenolic and flavonoidal compounds had been isolated from different parts of *C. procera*^{14&15}. Interestingly, molecular docking studies had been performed on some of these compounds to determine their inhibition effect of proteins responsible for the replication of SARS-CoV2 (severe acute respiratory syndrome coronavirus 2) which causes Coronavirus disease 2019 (COVID-19). For example, quercetin-3-O-rutinoside (rutin), hesperidine, and kaempferol-3-O-rutinoside (nicotiflorin) inhibit the main protease 3CLpro of SARS-CoV-2^{4&5}. Isorhamnetin-3-O-rutinoside (narcissin) showed a significant binding stability with receptor in the N3 binding site in the COVID-19 main protease⁶. Naringin can act as an inhibitor of main protease enzymes of SARS-CoV2⁷. Additionally, chlorogenic acid was suggested to weaken adsorption of SARS-CoV to cells and reduces its ability to infect surrounding tissues⁸.

The effect of solvent concentration on the TPC and TFC of *C. procera* hadn't been studied before. So, in this study we use different concentrations of methanol for extraction of *C. procera* and then determine TPC and TFC. The study was aimed to evaluate the effect of solvent concentration on TPC and TFC in different extracts of aerial parts of *C. procera* (leaves, stems and fruits). TPC and TFC were estimated by Folin Ciocalteu colorimetric assay and aluminum chloride method using tannic acid and quercetin as standards, respectively^{16&17}. Significant variation in TFC and TPC levels was observed in between the different samples.

C. procera presented highest both TPC and TFC in leaves extracted with 80% aqueous MeOH. In conclusion, the present investigation demonstrates that there is a significant effect of solvent concentration used for extraction of aerial parts of *C. procera* on their TPC and TFC contents.

MATERIAL AND METHODS

Chemicals

Tannic acid, sodium carbonate (7% w/v), sodium nitrite (5% w/v), AlCl₃ (10% w/v) and NaOH were purchased from Merck (Darmstadt, Germany). Quercetin, Folin-Ciocalteu's reagent were purchased from Sigma Aldrich (Tokyo, Japan).

Plant material

The fresh aerial parts (leaves, stems and fruits) of *C. procera* were collected in June, 2017 from El-Dakhla, New Valley, Egypt. The specimens were identified according to Tackholm¹⁸. The plant materials were shade dried at room temperature then ground into a fine powder. The specimen samples were deposited in the Herbarium of the Pharmacognosy Department, Faculty of Pharmacy, Assiut University, Assiut, Egypt with voucher number (0002015).

Extraction process

Equal quantities (50 g) of powdered leaves, stems and fruits were macerated with methanol and different concentrations of aqueous methanol (60%, 70% and 80%). After 48 hrs., samples were fine filtered through filter paper and the solvents were evaporated to obtain the methanolic extracts of the leaves, stems and fruits. These extracts were stored in a dry and cool place until needed for analysis.

Total phenolic content determination

The TPC was determined with the Folin-Ciocalteu's assay using tannic acid as a standard¹⁶. In the procedure, 0.5 mL of plant extracts were mixed with 1.5 mL of 10% (v/v) Folin-Ciocalteu's reagent (FCR), then after 5 minutes, 1.5 mL of 7% sodium carbonate solution was added. The final volume of the tubes was made up to 10 mL with distilled water and allowed to stand for 2hrs at room temperature. Absorbance of each sample was

measured spectrophotometrically (JENWAY 6300) against the blank at 765 nm. All the experiment was repeated three times and values are mean \pm S.D. and expressed in terms of phenol content (Tannic acid equivalent, TAE) per g of dry weight.

Total flavonoid content determination

The TFC was determined by Aluminium chloride method using quercetin as a standard¹⁷. In the procedure, 1mL of extract was mixed with 0.3 mL of 5% Sodium nitrite, then after 5 minutes, 3 mL of 10% Aluminium chloride was added. After 6 minutes incubation at room temperature, 2mL of 1M Sodium hydroxide was added. The final volume was made up to 10 mL with distilled water. Absorbance of samples were measured against the blank at 510 nm using a spectrophotometer (JENWAY 6300). All the experiment was repeated three times and values are mean \pm S.D. and expressed in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight.

RESULTS AND DISCUSSION

The study revealed that data of total phenolic contents was variable among both the different parts of *C. procera* and the different solvent concentrations. The highest phenolic content was found in leaves (12.96 mg Tannic acid/ g dry weight). Other parts of the plant such as stems and fruits had values of 2.04 and 4.1 mg Tannic acid/ g dry weight, respectively). Additionally, variation in the solvent concentration was evident in the TPC in the aerial parts of *C. procera*. TPC were high in leaves, stems and fruits extracts macerated with 80% aqueous MeOH (12.96, 2.04, 4.10 mg Tannic acid/ g dry weight, respectively) (Table 1).

As shown in table 2, the maximum flavonoid content was found in leaves (73.82 mg Quercetin/ g dry weight) followed by fruits (53.10 mg/g) then stems (27.47 mg/g). TFC were maximum in leaves macerated with 80% aqueous MeOH and in stems and fruits macerated with 100% MeOH (73.82, 27.47, 53.10 mg/g, respectively). Stems did not show a significant concentration of phenolic compounds nor flavonoids.

Table 1: Total phenolic content of plant parts of *Calotropis procera* (Ait.) R.Br. in different solvents.

Plant part	Total Phenolic Content (mg of Tannic acid equivalent/g of dry weight)			
	60% MeOH	70% MeOH	80% MeOH	100% MeOH
Leaves	10.71 \pm 1.29	9.09 \pm 1.32	12.96 \pm 1.27	9.46 \pm 1.68
Stems	0.66 \pm 0.06	1.83 \pm 0.18	2.04 \pm 0.11	0.14 \pm 0.10
Fruits	0.50 \pm 0.31	1.00 \pm 0.04	4.1 \pm 0.15	2.06 \pm 0.12

Table 2: Total flavonoid content of plant parts of *Calotropis procera* (Ait.) R.Br. in different solvents.

Plant part	Total Flavonoid Content (mg of Quercetin equivalent/g of dry weight)			
	60% MeOH	70% MeOH	80% MeOH	100% MeOH
Leaves	54.34 \pm 0.21	69.94 \pm 3.72	73.82 \pm 4.00	44.10 \pm 5.80
Stems	22.04 \pm 0.18	22.96 \pm 0.14	23.42 \pm 0.11	27.47 \pm 0.43
Fruits	21.92 \pm 0.07	26.66 \pm 0.14	23.16 \pm 2.04	53.10 \pm 3.88

Conclusion

The study revealed that the amount of phenolic and flavonoid contents varied not only from one part of the plant to another but also according to different solvent concentrations. It showed that the maximum TPC and TFC were found in leaves followed by fruits and stems. TPC was found highest in leaves, stems and fruits extracts macerated with 80% MeOH. While, TFC was highest in leaves macerated with 80% MeOH and in stems and fruits macerated with 100% MeOH. These results help in deciding the proper solvent concentration for extraction procedure of aerial parts of *C. procera* to obtain maximum contents of phenols and flavonoids, thus obtaining maximum biological activities.

Conflict of Interests

Authors do not have any conflict of interests

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تأثير تركيزات المذيبات على التقدير الكمي للمحتوي الفينولي والفلافونويدات الكلي للأجزاء الهوائية من نبات كالتوتروبسيس بروسيرا (أيت). أر. بي أر.

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تعتبر الفينولات والفلافونيدات الموجودة في النباتات الطبية من أهم المكونات النشطة بيولوجياً. يعتبر نبات كالتوتروبسيس بروسيرا (أيت). أر. بي أر (العائلة الدفلية) نبات طبي له نطاق واسع من النشاط الحيوي. الهدف من هذه الدراسة هو تقييم تأثير تركيز المذيب على المحتوى الفينولي الكلي (TPC) ومحتوى الفلافونويد الكلي (TFC) في المستخلصات المختلفة لأوراق وسيقان وثمار كالتوتروبسيس بروسيرا. تم تقدير TPC و TFC بواسطة مقايصة فولين سيوكالتو اللونية وطريقة كلوريد الألومنيوم باستخدام حمض التانيك والكيرسيتين كمعيارين، على التوالي. وقد أظهرت هذه الدراسة تبايناً في مستويات TPC و TFC بين العينات المختلفة. أظهر نبات كالتوتروبسيس بروسيرا أعلى تركيز لكل من TPC و TFC في الأوراق المستخلصة باستخدام ٨٠% ميثانول مائي. يوضح البحث الحالي أن هناك تأثيراً كبيراً لتركيز المذيب المستخدم لاستخلاص الأجزاء الهوائية من كالتوتروبسيس بروسيرا على محتويات TPC و TFC.