Research Article

Fluorescence Analysis, Extractive Values and Cytotoxic Screening of *Crataeva adansonii* DC Leaf and Bark through Brine Shrimp Larvae Assay

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Abstract | *Crataeva adansonii* is a medicinal plant and many folklore uses of this plant are well known to us. Pharmacognostic studies like finding extractive values and nutritional values play a vital role in the determination of adulterated or exhausted drugs. It lays down parameters for authentication and standardization of drugs. The cytotoxic study helps in the evaluation of a drug for its toxicity, which is determined by the prevention of growth and replication. Such drugs can be used to treat cancer, multiple sclerosis and rheumatoid arthritis. During the present study ethanolic extracts of leaf and bark parts of *C. adansonii* were evaluated through cytotoxic activity. Brine shrimp larvae assay was conducted for this purpose. Extractive values of leaf and bark parts were estimated using n-hexane, chloroform, ethanol, methanol, water, and ether as a solvent. 10g of plant powder was dissolved in 200 ml solvent during various extract preparations Fluorescence study was conducted after treating the powder drug with different reagents including ethanol, methanol, acetic acid, n-hexane, HCl, nitric acid, carrageenan, glacial acetic acid, diethyl ether and picric acid and change in coloration was observed. Distinct fluorescence was exhibited by the drug under the normal daylight and UV-radiations of short wavelength (256nm) and long wavelength (365nm). Both the leaf and bark parts showed maximum extractive values in water (23% and 15% respectively) followed by methanol (15% and 6% respectively. The leaf part showed greater extractive values as compared to the bark. The cytotoxic study revealed that the ethanolic extract crude drugs of each part dose-dependently exhibited cytotoxic activity. At higher concentration (100-1000 µg/ml) the leaf and bark ethanolic extracts showed cytotoxicity and at lower concentration (10 µg/ml) very low cytotoxic effect was observed (Figure 4). The LC_{50} values for crude leaf extract were found to be 5.34 and for that of stem bark extract, it was 7.44 ug/ml. This study manifests that the selected plant can be helpful in the preparation of novel pharmaceuticals. The pharmacognostic evaluation of this important medicinal drug will be supportive in the detection of adulteration and drug quality control.

Received | July 13, 2020; **Accepted** | August 18, 2020; **Published** | September 01, 2020
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Citation | Bibi, S.F. and S. Din. 2020. Fluorescence analysis, extractive values and cytotoxic screening of *Crataeva adansonii* DC leaf and bark through brine shrimp larvae assay *Sarhad Journal of Agriculture,* 36(3): 949-957.
DOI | http://dx.doi.org/10.17582/journal.sja/2020/36.3.949.957
Keywords | *Crataeva adansonii*, Fluorescence study, Extractive values, Cytotoxicity analysis, Physicochemical characters, Pharmacognostic study

Introduction

Crataeva adansonii is a moderate-sized deciduous tree belonging to family Capparaceae (Ryan and Ray, 2004). It is commonly known as Varun, temple plant and sacred garlic pear. It grows well on sandy loam in arid areas. In Pakistan, it is found on the Sub-Himalayan track. Its leaves are trifoliate compound (Figure1; Hoang, 2013). The fruit is globose in shape having a 4-5cm diameter. The flowers are showy

and white, bearing long purplish stamens (Figure 2; Jeevan, 2016). Due to the elongated appearance of stamens, the plant is often called a spider tree.

Figure 1: *Crataeva adansonii tree.*

Figure 2: *Crataeva adansonii flowers.*

Traditionally, this plant is used as diuretic, astringent, antipyretic, anti-inflammatory, appetizer, liver stimulant, contraceptive, stomachic, demulcent and tonic. Use of *C. adansonii* against bronchitis, calculi, asthma, cough, renal and urinary troubles, dyspepsia and uterine infections is also well known in traditional medicine (Kumar *et al.*, 2012; Gitte *et al.*, 2012). Previously anticancer, antimicrobial, antihypertension, analgesic, antiparasitic, antidiabetic and antioxidant activities of *C. adansonii* have been reported (Zingue *et al.*, 2016; Adjagba *et al.*, 2017). Cytotoxic studies have been carried out in various genera of the family Capparaceae including *C. nurvala* (Sinha *et al.*, 2013; Ali *et al.*, 2014; Hade *et al.*, 2016; Parvin *et al.*, 2011) and *C. nurvala* (Da Silva Ferreira *et al.*, 2013).

The extractive value of a crude drug refers to its soluble or extractable material in a suitable solvent. The solution obtained after the extraction of a drug contains different phytoconstituents. The composition of those constituents depends on the nature of the solvent and nature of a drug, it also gives an idea of whether the crude drug is genuine or exhausted (Tatiya *et al.*, 2012). Extractive values play an imperative role in crude drug evaluation. During extraction, the use of different solvents assures exhausted material and adulteration e.g water and alcohol are used to detect poor quality signs, defective processing and presence of adulterants in the drug. Lipid content of the crude drug is indicated by extractive values, using petroleum ether as a solvent (Kokate, 1994; Madhavan *et al.*, 2009). The physicochemical standards and Preliminary phytochemical analysis help in the detection and identification of adulteration in the drug. Closely related species of the same family and genus can also be distinguished through these findings (Gavit and Patel, 2019). The values of the total yield of extracts are resultants of the effects of the extraction condition. With the increase in the polarity of the solvents the extraction yield is increased (Ranjith, 2018).

Fluorescence analysis helps in the determination of constituents and it is effectively sensitive (Prakash and Vedanayaki, 2019). Many drug constituents show fluorescence in the visible range of daylight while others exhibit it under ultra-violet light. The same material appears to be dissimilar in light of different wavelengths, some substances are not luminescence in visible light but they show fluorescence when observed under UV light. The fluorescence quality of any drug supports characteristic highlights for drug assurance due to its exceptional specificity (Sharma and Dhanawat, 2019). If fluorescence is not shown by a drug then the application of different reagents to their derivatives or decomposition products may make them fluorescent. This analysis can be used as a qualitative assessment for drug standardization. During the identification of various crude drugs, fluorescence can serve to be a fingerprint (Ansari *et al.*, 2006; Reddy and Chaturvedi, 2010). UV light qualitative evaluation of crude drugs is performed which is a distinguished parameter regarding the pharmacognostic evaluation of drugs (Reddy and Chaturvedi, 2010). Different plant material gives

different coloration when treated with various chemicals and solvents (Sumithra and Kumar, 2016; Suriyavathana *et al*., 2018). The fluorescence quality of any drug supports characteristic highlights for drug assurance due to its exceptional specificity (Sharma and Dhanawat, 2019).

Toxic compounds are found in the extracts of some medicinal plants (Iqbal, 2016). For safety assessment, the toxicity profiling of these plants is very effective (Chan *et al*., 2016). *Artemia salina* (Brine shrimps) are widely used during the cytotoxicity experiments to check the potential of various chemical entities. Phytomedicine is used against cancer and other diseases (Amara *et al*., 2008). Almost half of the anticancer drugs are derived from plants (Newman and Cragg, 2007). Brine shrimp's larvae are used for evaluating the cytotoxic potential of plant-derived drugs during anticancerous activity. Shrimp's larvae can respond in quite a similar manner to the mammalian's carcinoma (Solis *et al*., 1993). Therefore, cell lines assay can be replaced by brine shrimp assay for cytotoxicity as it is easily achievable and less costly (Piccardi *et al*., 2000). Plant-derived biologically active compounds show toxicity towards *Artemia salina*. This quick and economic activity is established for fractionation and screening process (Carballo *et al*., 2002). The role of *Artemisia salina* in a marine ecosystem cannot be neglected. It is an invertebrate, also known as brine shrimp. Brine shrimp lethality experiment helps find medium lethality concentration LC_{50} calculated for toxins and plant extracts (Lagadic and Caquet, 1998). The lethality experiment of brine shrimps as described in the literature is considered as a useful tool to detect cytotoxicity caused by heavy metals, plant extracts and toxins produced by cyanobacteria (Sharififar *et al*.,2009).

In the present research, the ethanolic extract of leaf and bark parts of *C. adansonii* was investigated to find its cytotoxic potential against brine shrimp larvae. Powder drug of the plant was analyzed during the study of fluorescence and extractive values.

Materials and Methods

Collection of plant material

C. adansonii DC. leaf and bark were collected from the University of Peshawar campus and identified by plant taxonomist Prof. Dr. Siraj-ud-Din. A voucher specimen was submitted in the herbarium of the University of Peshawar (PUP).

Preparation of crude extract

Leaf and bark parts of *C. adansonii* were collected and cleaned thoroughly, this material was shade dried and ground to make a fine powder. One kilogram plant powder was soaked in 5-6 liters of ethanol. The mixture was kept in an airtight jar for 12-15 days at room temperature and occasional vigorous shaking was ensured. This solution was filtered and then it was concentrated in a rotary evaporator to get a thick crude extract. After repeating this process 3 times the collected plant extracts were preserved in a cold and dry place (Miliauskas *et al*., 2004).

Extractive values

Various solvents can be used to extract chemical constituents of a powder drug, for example, alcohol extracts resins and tannins; water extracts mucilages and glycosides; ether extracts oily and fatty substances. The amount of an extract is the approximate measure of its chemical constituents. The number of adulterants can be calculated by these values (Kokate, 1994).

Procedure

Extractive values were calculated for the drug, using standard protocols (Ansari *et al*., 2006; Yadav *et al*., 2007). 200 ml each of various solvents were used to dissolve 10 g of crude powder drug and kept in airtight bottles for 7 days with occasional vigorous shaking. The solution was strained through a muslin cloth and then filtered using a filter paper. A rotary evaporator was operated to evaporate the filtrate. After repeating the process three times, extracts were combined and weighed, their percent extractive values were calculated using the following formula:

$$\text{Percent (\%) extractive value } \left(\frac{W}{W}\right) = \frac{\text{Weight of dried extract}}{\text{Weight of plant material}} \times 100$$

Fluorescence study

Certain drugs can show different kinds of fluorescence when they are exposed to daylight and also under ultraviolet radiation, so the fluorescence technique can be used for crude drugs as an identification tool (Jarald and Jarald, 2007). During this research study, the methodology of Kokoshi *et al*. (1958) was followed to study the fluorescence of *C. adansonii* leaf and bark.

Procedure

Fluorescence analysis was carried out using crude powder drugs as such and also after treating it with

different reagents including ethanol, methanol, acetic acid, n-hexane, HCl, nitric acid, carrageenan, glacial acetic acid, diethyl ether and picric acid. The short and long wavelengths of UV light and daylight were used to observe the samples (Brain and Turner, 1975; Chase and Pratt, 1949; Evans, 2002).

Cytotoxicity

Ethanolic extracts of *C. adansonii* DC. leaf and bark were used to conduct brine shrimp assay to determine their preliminary cytotoxic potential by following the methodology proposed by Atta-ur-Rhman *et al.* (2001).

Technique for hatching

A hatching tray (22 x 32 cm) was partitioned with the help of a perforated plate into two chambers; brine solution was poured into the tray to half fill it. In one chamber 25 mg *Artemisia salina* eggs were sprinkled and covered with black paper. A lamp was suspended over the other chamber of the tray to illuminate it and the apparatus was kept at room temperature. After a hatching period of almost 60 hours, brine shrimp napualli were mature, and they moved from the dark chamber towards the enlightened one across the perforated partition.

Preparation of sample

A stock solution was prepared by dissolving 20 mg of plant extract in a 2 ml solvent. 5, 50 and 500 µl of this solution was transferred to glass vials using a micropipette. In this way, 3 replicates were prepared for each concentration i.e. 10, 100 and 1000 µg/ml. Each vial was kept open overnight to evaporate it and then a 5ml sea salt solution was poured into it. 10 mature napualli were shifted to each vial using Pasteur pipette. For positive and negative control, reference cytotoxic drug and brine solution were used respectively. Vials were kept at room temperature in light.

Calculations and statistical analysis

The number of dead and alive napualli was recorded after 24 hours. LC_{50} values were determined considering a 95% confidence interval with the help of the Finney computer program (Ibrar and Muhammad, 2011; Khan *et al.*, 2010).

Results and Discussion

Extractive values

Extractive values are valuable for uncovering exhausted or adulterated drugs. Extraction with different solvents may give a clue about the adulteration of different kinds of exhausting materials (Madhavan *et al.*, 2009). Extractable values with alcohol and water are indicative of the existence of adulterants, in addition to any defects in processing and low quality of the drug. On the other hand, extraction with petroleum ether shows the presence of lipid contents (Kokate, 1994).

During this research, percent extractive values were determined for bark and leaf of *C. adansonii* using various solvents, including n-hexane, ethanol, chloroform, distilled water, acetone and butanol. Maximum extractive value for the leaf part was found in water (23 %) followed by methanol (15 %), similarly, in the case of the bark part, it was water (15 %) followed by methanol (6 %). Extractive values in other solvents were variable (Table 1; Figure 3).

Table 1: *Percent extractive values of Crataeva adansonii leaf and stem bark with different solvents.*

S. No	Plant part	Solvent	Extractive value (%)
1	Leaf	Methanol	15
		Ethanol	11
		N-hexane	4
		Water	23
		Chloroform	6
		Ether	3
2	Bark	Methanol	6
		Ethanol	3
		N-hexane	3
		Water	15
		Chloroform	4
		Ether	2

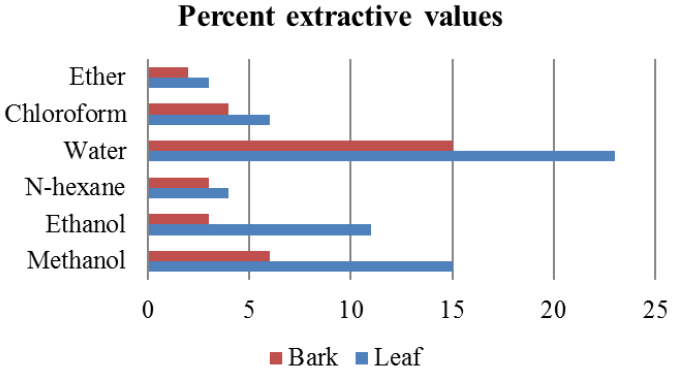

Figure 3: *A comparison of extractive values of Crataeva adansonii leaf and stem bark.*

Prakash and Vedanayaki (2019) used various solvents

like ethyl acetate, aqueous, acetone, hexane, methanol and chloroform to extract *Zephyranthes citrine* plant powder. The highest quantity of yield was shown by methanol i.e. 4.00g. During the physicochemical analysis of *Amaranthus blitum* maximum extractive value was found in water (24.43 ± 0.983). Ethanol and methanol also showed considerable extractive values of 10.66 ± 0.666 and 10.46 ± 0.290 respectively (Gavit and Patel, 2019). Chloroform, ethyl acetate, petroleum ether, methanol, ethanol, water and acetone were used for the maceration process and extraction of *Murraya koenigii Psidium guajava* and *Curcuma longa*. The yield of aqueous extract was maximum (26.46%) due to the increased polarity of water. The extractive value of methanolic extract was slightly lower (16.42%) than ethanolic extract.

Fluorescence study

The presence of diverse chemical constituents in the plants accounts for variable fluorescence phenomena under UV light and in regular daylight. In agreement with the previous work, *C. adansonii* stem bark and leaf powder were subjected to fluorescence analysis using several reagents including ethanol, methanol, acetic acid, n-hexane, HCl, nitric acid, carrageenan, glacial acetic acid, diethyl ether and picric acid (Table 2). The powder drug was treated with the reagents and then exposed to ordinary daylight, UV- light of short wavelength (255nm) and long wavelength (366nm). Leaf and bark powders manifested different colors when they were exposed to the radiation. The crude methanol extract and powder drug of *Zephyranthes citrina* were analyzed during a fluorescence study after mixing with various reagents (Prakash and Vedanayaki, 2019) and various colors were recorded. Sharma and Dhanawat (2019) studied the fluorescence of *Euphorbia neriifolia* Linn. leaf and stem and declared it as an important parameter for herb standardization. Similarly, Azhagumadhavan *et al.* (2019) evaluated fluorescence analysis and other Physico-chemical parameters of *Costus spicatus* rhizome and described various diagnostic characters. Under UV and ordinary light, the powdered drug and extract showed reproducible and distinctive color change. Fluorescence analysis of *Murraya koenigii Psidium guajava* and *Curcuma longa* was performed after treating with distilled water, ethanol acetone, diethyl ether, benzene, methanol, chloroform, glacial acetic acid, nitric acid, sulphuric acid, hydrochloric acid, 1N NaOH, 5% picric acid, 5% FeCl3, 1N NaOH + methanol, and the solutions were observed for their characteristic colors under the visible daylight and long and short wavelength UV light (Ranjith, 2018). Fluorescence analysis is one of the diagnostic tools used for the detection of adulterants in whole plants as well as in their powder forms.

Table 2: *Fluorescence analysis of Crataeva adansonii leaf and stem bark.*

S. No	Part	Solvent	Daylight	UV-255	UV-366
1	Leaf	Powder	Olive green	Brown	Blackish
		Ethanol	Green	Medium brown	Brownish black
		Methanol	Mustard green	Brown	Bluish
		Acetic acid	Olive	Green	Purplish green
		N-hexane	Medium olive	Light green	Purplish green
		HCl	Olive	Olive green	Purplish brown
		Nitric acid	Brown	Mustard	Reddish-brown
		Carrageenan	Green	Dark brown	Blackish
		Glacial acetic acid	live	Green	Chocolate brown
		Diethyl ether	Olive	Purple Green	Dark purple green
		Picric acid	Pale olive	Yellowish green	Dark brown
2	Bark	Powder	Yellowish-brown	Brown	Dark brown
		Ethanol	Pale brown	Brown	Brown
		Methanol	Yellowish-brown	Brown	Dark brown
		Acetic acid	Light pale brown	Mustard	Purplish brown
		N-hexane	Light pale brown	Olive green	Light brown
		HCl	Light brown	Light brown	Light purplish brown
		Nitric acid	Reddish-brown	Yellowish green	Dark reddish-brown
		Carrageenan	Light brown	Brown	Dark brown
		Glacial acetic acid	Light brown	Yellowish green	Brown
		Diethyl ether	Brown	Brown	Dark brown
		Picric acid	Yellow	Pale	Light brown

Cytotoxic study

The cytotoxic activity of *C. adansonii* crude extract was compared with standard drug etoposide (standard cytotoxic drug). At higher concentration (100-1000 µg/ml) the leaf and bark ethanolic extracts showed cytotoxicity and at lower concentration (10 µg/ml) very low cytotoxic effect was observed (Figure 4). The LC_{50} values for crude leaf extract were found to be 5.34 and for that of stem bark extract, it was 7.44 ug/ml. Stem bark extracts *of Albizia lebbeck* were evaluated for cytotoxic activities of the synthesized nanoparticles on human breast cancer cell lines, the extract was found to be effective (Umar *et al*., 2019). The methanolic stem and leaf extracts of *Cynometra ramiflora* were tested on brine shrimp nauplii, as a result strong lethality was demonstrated in preliminary cytotoxicity assay (Afrin *et al*., 2019). During a similar cytotoxic activity, the crude methanolic extract of *Bougainvillea glabra* leaves was screened in a lethality bioassay by using brine shrimp nauplii (*Artemia salina*) and it was revealed that even at low doses the extracts were toxic (Dokuparthi *et al*., 2018). Cytotoxicity screening data offer significant primary data to help choose plant extracts with possible antineoplastic attributes for future work (Itharat *et al*., 2004). The cytotoxicity results obtained from the study on leaf and bark of *C. adansonii* may offer potential avenues for the development of cytotoxic drugs.

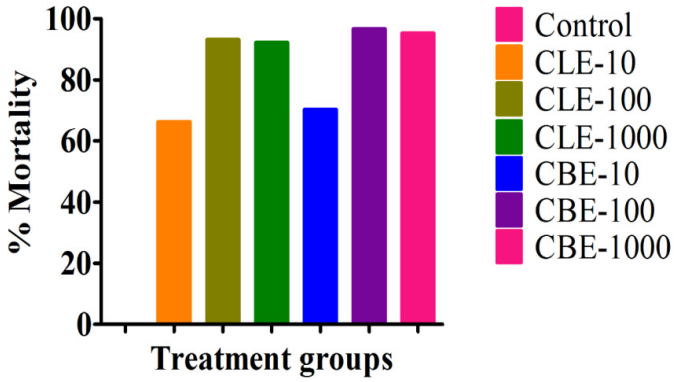

Figure 4: *Cytotoxicity of ethanolic extract of Crataeva adansonii leaf and bark.*

Conclusions and Recommendations

From this study, it is concluded that *C. adansonii* leaf and bark ethanolic extracts show significant cytotoxicity in a dose-dependent manner. Evaluation of standardizing parameters for drug preparation was an important part of this study. Extractive values and fluorescence study is supportive in the detection of adulteration and drug quality control. The extractive value of the formulation helped find the most effective solvent for extraction and to determine the characteristics of its chemical constituents. It is recommended that further studies may be carried out to check the pharmacological potential of the drug.

Novelty Statement

This research work discovers the cytotoxic potential of ethanolic crude extract of *C. adansonii* and highlights that the drug can be helpful in the preparation of novel pharmaceuticals. Moreover, the parameters for authentication and standardization of drugs are laid down through finding extractive values and fluorescence study of the drug.

Author's Contribution

This paper is a part of the major author's contribution to the requirement of Ph.D. Syeda Farzana conducted the experimental work and Prof. Dr. Siraj-ud-Din guided her as a research supervisor during the entire research process, data compilation and analysis.

Conflict of interest

The authors have declared no conflict of interest.

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