Applied Biological Research 25(1): 29-35; (2023)
DOI: 10.5958/0974-4517.2023.00003.4

ETHNOMEDICAL USE OF *Calotropis procera* (Aiton) Dryand. IN KADUNA STATE OF NIGERIA AND BIOLOGICAL EVALUATION

Mahmoud Dogara Abdulrahman

Biology Education Department, Faculty of Education, Tishk International University, Erbil, Kurdistan Region, Iraq
*****e-mail: *abdulrahman.mahmud@tiu.edu.iq; abdouljj@yahoo.com*

(Received 28 August, 2022; accepted 31 December, 2022)

ABSTRACT

The development of current healthcare system is greatly influenced by plants having therapeutic potential, especially the medicinal plants. The advent of new ailments worldwide has encouraged the researchers to explore the potential of different plant parts as well. The research aims to compile information on *Calotropis procera* traditional usage and biological activity from a native population in the northern Nigeria, Kaduna state. Random sampling was employed for the collection of traditional medicinal knowledge on *C. procera.* Radical scavenging activity, ferric reducing antioxidant power, and disk diffusion assay was employed to evaluate its biological activity of ethanol and aqueous extracts of *C. procera*. The study revealed that *C. procera* is traditionally used in the area to treat a variety of ailments like hypertension, migraine, and spiritual problems having the highest fidelity at 0.9%. The leaves exhibited highest radical scavenging, ferric reducing power, and antibacterial activity at 86.01 µg ML^{-1} and 18.1 mmol g^{-1}. The largest zone of inhibition against Pseudomonas aeruginosa was found in an ethanol leaf extract of *C. procera* at 23.5 mm. The study revealed the leaf of *C. procera* as a good avenue for the development of modern drugs.

Keywords: Antioxidants, antimicrobial, *Calotropis procera*. respondents, traditional knowledge

INTRODUCTION

The medicinal plants since their discovery have widely been used as therapeutic medicines with significant commercial significance (Mahmoud *et al.*, 2020). In past, several diseases were treated with medicines derived from plants (Jeruto *et al.*, 2008). Therapeutic plants are now widely used in the field of human health care (Kankara *et al*., 2022). Traditional African communities place a great value on and frequently employ phytotherapy to cure many ailments, so plants serve as their primary healthcare source (Abo *et al.*, 2008). Today, more people are turning to natural remedies (Kayfi *et al*., 2021). Human bodies are intricately designed by nature to house a wide range of cell types, each with its own unique set of chemicals. Molecules consist of a variety of atoms bound together through chemical bonds (Yunusa *et al*., 2018). The bonds between the atoms do not usually break in such a way that the molecules are left with a single unpaired electron. On the other hand, when a weak link is broken, free radicals are produced (Abdulrahman *et al.*, 2019). Drug resistance is a growing problem in modern medicine as harmful bacteria adapt to the wide variety of medications used to treat them (Abdulrahman *et al.*, 2019). Antibiotic resistance is a significant issue in modern medicine, and as a response, interest in historically used herbs that exhibit promise as therapeutic remedies has increased. Thousands of useful chemicals with therapeutic potentials are found in medicinal plants. Without the assistance of medicinal plants, modern healthcare infrastructure would not exist. Plants with therapeutic potential are now widely accepted and acknowledged, and they are still the only practical choice (Dogara *et al.*, 2021). Researching Nigeria's rich flora has the potential to yield a novel therapeutic agent. Therefore, study of underutilized Nigerian flora is required to develop new medicines. The genus *Jatropha*, which is part of the family Euphorbiaceae, has about 170 species (Umarkar *et al*., 2011). The *Calotropis procera* plant serves manifold-purposes including therapeutic applications (Umarkar *et al.*, 2011). It is impossible to overstate the value of ethnobotany if standardized in vitro and in vivo techniques are available to support claims made by traditional herbalists. The purpose of this research was to compile information on *C. procera* traditional usage and biological activity from a native population in the Kaduna state of northern Nigeria.

MATERIALS AND METHODS

Study area

An ethnobotanical study was done from June 2021 to June 202 in the northern Nigerian state of Kaduna (10° 35' N, 7° 19' E), which has a population of 6,066,562 and covers 46,056 km^2. Even though weather changes from season to season, the area has two main types of weather: dry (October to May) and wet (June to August). Nomadic Hausa and Fulani tribes make up most inhabitants (Abdulrahman *et al.*, 2022). They mostly work as government employees, farmers, animal-husbandry, fishermen, and hunters.

Sampling and interview sessions

Methods of expert and random sampling were used. An unstructured interview format was adopted. Pre-study questionnaire validation was performed. From November 2021 to June 2022, we conducted in-depth interviews with traditional herbalists and other individuals possessing traditional expertise to compile the data for this study. Each respondent was follow-up three or four times to guarantee the accuracy of the data gathered (Age, education, occupation, practicing the various traditional use of *C. procera* and for how long? other uses of plant, plant part used, the method of preparation, method of administration, nature of plant whether wild or cultivated or both? is any part of the plant toxic?, etc.). There was no trust placed in the information that contradicted what had been learned on earlier visits. Information was collected by speaking to locals in their own tongue.

Ethnomedical data analysis

Excel 2016 was used to compute the descriptive analysis. The frequencies and percentages were determined using the respondents' sociodemographic data as follows: gender, level of education, occupation, diseases treated, plant component utilized, administration method, preparation method, experience (Abdulrahman *et al.*, 2008). Fidelity level (FL) was calculated as follows:

$$\text{Fidelity level (FL)} = \frac{\text{Ns}}{\text{N}} \; x\ 100$$

wherein Ns is the total number of respondents treated for ailment by the plant, while N is the overall number of respondents who mentioned the plant as treatment to different ailments (Abdulrahman *et al.*, 2018).

Plant collection identification

The plant samples of *Calotropis procera* were collected from outdoor spaces, field and backyard gardens, and herbarium specimens were created. The plants were deposited at the Ahmadu Bello University Herbarium in Zaria after getting their identify confirmed by a trained taxonomist under voucher number V/N ABU900218.

Extraction of plant samples

Collected leaves, whole plant, and the root were washed with running water to remove dirt, stains, and latex. Plant samples were powdered, and 100 g sample was extracted by Soxhlet technique using aqueous and ethanol solvents separately and extract was filtered through Whatman filter paper No. 2.

Both ethanolic and aqueous plant components were put through an evaporation process to obtain crude extract. For the ethanolic and aqueous extracts, the vacuum was set at 40ºC, and the pressure of solvent was 300 and 72 pascals respectively, respectively. The extract yield was achieved by leaving the crude extract produced from the leaves, whole plant, and root in an oven for an entire night.

6.4.1.2 1, 1-Diphenyl-2-picryl hydrazyl (DPPH) Radical Scavenging Activity

The effects of 100 g mL^{-1} (0.004% w/v) DPPH methanolic solution on seven various concentrations of leaves, whole-plant, and root of 100% ethanol and 100% aqueous were tested. The solution was maintained at room temperature for 30 min in dark. Quercetin was used as a benchmark for comparison (Abdulrahman *et al*,, 2019). The formula used to determine the amount of radical scavenging activity was:

$$\% \text{ Inhibition} = \frac{\text{Bo} - \text{B1}}{\text{Bo}} \ x\ 100$$

Where B1 is the sample absorbance and Bo is the control absorbance reaction**.**

Ferric reducing antioxidant power (FRAP) assay

Acetate buffer (B, 300 mM), 2,4,6-tri (2-pyridyl) –S-triazine (10 mM) TPTZ in HCl (40 mM), and $FeCl_3$ (20 mM) were mixed in a 10:1:1 ratio and heated in a water bath at 37°C for 10 min. Then, 285 μL of FRAP working solution (100 g mL^{-1} concentrations) was added to 15 μL ethanol or aqueous (100%) extracts of leaves, whole plant, and root samples and incubated at room temperature for 30 min in dark (Abdulrahman *et al.*, 2019).

Antimicrobial evaluation

Microbial inoculum (200 µL) containing 1.106 (CFU) mL^{-1} were planted onto solidified mueller-hinton plates. To 20 µL of 1000 g mL^{-1} plant components (ethanolic and aqueous) extract, Whatman No. 1 filter paper discs (6 mm) were impregnated. Using sterile forceps on the plates, the impregnated disc was inserted. The plates were incubated at 37°C for 24 h (Abdulrahman *et al.*, 2019). Measurements of the inhibitory zone were made with a ruler, and all experiments were repeated three times.

***Principal component analysis* (PCA)**

Analysis of the correlation matrix between the variables using principal components. The goal is to analyze pairs of variables that are linearly connected (Yunusa *et al*., 2018).

Statistical analysis

For statistical analysis of data Statistical Analysis System (SAS) for data analysis (University version 9.4) was used, the experiments were conducted in a completely randomized design with each treatment replicated three times. Duncan's multiple range test was used as a post-hoc test to determine whether there were statistically significant variations between groups' means at the $p \leq 0.05$ level following a one-way repeated-measures analysis of variance (Abdulrahman *et al.*, 2019).

RESULTS AND DISCUSSION

Demographic profile of respondents

We administered an in-depth questionnaire to a random sample of 98 respondents (71.43% male and 28.57% female). There were 30 respondents in the age of 30 and 49 (19 men and 11 women), 55 respondents in the age of 50 and 69 (41 men and 14 women), 12 respondents in the age range of 70 and 89 (10 men and 2 women), and 1 male was older than 90 years. The information acquired through interviews aids in the documentation of authentic traditional knowledge and serves as a reference for scientific study (Ijaz *et al.,* 2016). Informants play a significant role in such ethnobotanical studies. Their age, gender, education level, and occupation offer important context for the survey and make it easier to analyse and understand the findings in a more realistic social context (Merouane *et al*., 2022).

Diseases treated with C. procera

Most of the population in Nigeria historically uses plant-based therapies, and traditional medical knowledge is a component of Nigerian culture. Quite many diseases were treated with different parts of *C. procera* (Table 1). All parts of the plants (Fig. 1) were mentioned to be very beneficial, and respondents used them to give their patients for their wellbeing (Table 1). Secondary metabolites accumulated in various parts of plants are the main reasons for the medicinal potential of medicinal plants (Ijaz *et al*., 2016). As previously mentioned, plants with a high fidelity value are reliable signs that they contain the substances having high therapeutic potential (Abdulrahman et al., 2018; Dogara et al., 2021). Respondents reported that 41.12 % of the species were collected from the wild.

Table 1: Fidelity level of *C. procera* against various diseases in Kaduna state, Nigeria

Diseases	Method of preparations and administrations	Fidelity level (%)
Arthritis	Place 10 fresh leaves in a small pot with 1 litre of boiling water for 15 minutes, then strain and drink extract.	0.5
Anti-aging	11 leaves be soaked at night, and one drink be taken in the morning.	0.7
Asthma	A cup of flowers be boiled in 2 L water and consume the extract three times a day.	0.6
Breast cancer	Take once daily, preferably in the morning after breakfast, and mix 1 cup of fresh root with 1 teaspoon of honey in a small pot. Add 2 cups of water and simmer for 5 min with low heat.	0.6
Diabetes	Drink boiled water with a tablespoon of powdered leaves, root, or whole plant thrice a day.	0.8
Energy	Eating fresh leaves directly	0.5
Eczema	Vaseline be mixed with freshly cooked leaves, then applied consistently to the entire body until it disappears.	0.7
Hypertension	Cup of warm water, add fresh leaves and tablespoon of dried root, leave it for 30 min, and drink once a day after meal.	0.9
Heart problem	Boiled water with tablespoon of powdered leaves or whole plant and drink twice in a day.	0.5
Migraine	Vaseline be mixed with freshly cooked leaves, then applied consistently to the affected area until it disappears.	0.9
Measles	Vaseline be mixed with freshly cooked leaves, then applied consistently to the entire body until it disappears.	0.7
Stomach pain	Apply the powdered leaves all over the stomach until disappear.	0.5
Spiritual	Take new fresh leaves from the top part of the plant and consume freshly every day until cured	0.9

Note: S/N = Serial Number

Fig. 1: *Calotropis procera* collected from the wild

Biological evaluation of C. procera

One of the popular techniques used by many researchers to assess the antioxidant capacity of a certain plant component extract is DPPH (Sultana *et al.*, 2009). The technique is reliable and has the potential to convert a DPPH free radical into a nonradical state by accepting an electron from the evaluated antioxidant plant extract. The electron uptake from the antioxidant sample enables the technique to decolorize from purple to yellow (Abdulrahman et al., 2019). DPPH was used to test the antioxidant capacity of the ethanolic and aqueous extracts of leaves, whole plant, and root of *C. procera.* All parts exhibited antioxidant activities with both ethanolic and aqueous leaf extract showing the highest activity (Table 2). The polarity of metabolites and the nature of chemicals present substantially influence the activity of distinct *C. procera* sections. To ascertain the reducing ability of plant extracts, the ferric reducing antioxidant power was assayed. When compared to the scavenging activity of the studied plant components, the ethanolic and aqueous extracts did not significantly differ in their antioxidant status/reducing ability (Table 2). The variation observed in DPPH and FRAP of *C. procera* may attributed to behaviour of phenolic compounds or reaction with the assay's design. Similar observations have previously been reported in a comparative evaluation of antioxidants based on several in vitro assays (Dudonne et al., 2009). The plant *C. procera* helps in preventing the colon cancer in a rat model by reducing the oxidative stress and inflammation (Kumar et al., 2022). The antioxidant potential of methanol extract of both plants has been found to be greater than that of any other extract tested (Rani *et al.*, 2019).

Table 2: Evaluation of ethanolic extract of *C. procera* for antioxidant and antibacterial activities

Plant part (extract) ($\mu g\ mL^{-1}$)	% of inhibition of DPPH ($\mu g\ mL^{-1}$)	% of inhibition FRAP Fe^{2+}/ (mmol g^{-1})	Zone of inhibition (mm) (*Pseudomonas aeruginosa)*
Leaves (ethanol)	86.01^{a}	16.5^{b}	23.5^{a}
Whole plant (ethanol)	73.02^{c}	15.1^{d}	19.8^{b}
Root (ethanol)	80.04^{b}	16.3^{b}	22.9^{a}
Leaves – Aqueous	85.03^{a}	18.1^{a}	11.0^{c}
Whole plant (aqueous)	80.03^{b}	16.4^{b}	13.3^{d}
Root (aqueous)	69.01^{d}	15.9^{b}	15.2^{e}
Quercetin	70.04 ^{c,d}	15.8^{b,c}	-

Note: The values represent the means standard deviations of three independent experiments performed three times each. Similar alphabets vertically are not statistically different at the p≤ 0.05 level.

The plant parts appeared to have broad spectrum effects against *Pseudomonas aeruginosa* with ethanolic leaf extract depicting the largest zone of inhibition (Table 2). Maximum antibacterial activity was observed with aqueous extract of *C. procera* against all the test bacteria (Rani *et al.*, 2019). The study showed that plant activity differed depending on the solvents used and the parts of the plant involved. To that end, a range of solvents and concentrations must be used to ascertain the biological activity of a particular plants (Abdulrahman, 2021). It appears that the high activity of leaf extract is due to the solvent's superior ability to extract the compounds from leaves, as well as the fact that the leaves are primary makers of secondary metabolites before they are transmitted to the other plant parts. Multivariate analysis is a good way to reveal the difference between closely related biological activities (Jamalova *et al.,* 2021). Principle component analysis was used to better understand the composition of the plants (Kinda *et al*., 2020). The unsupervised PCA analysis model makes it possible to divide extracts into clear groups based on the activity of the solvent (Fig. 2). To establish a connection between the various biological activities of *C. procera* features, principal component analysis with loadings were conducted. The PCA showed a close connection between the aqueous leaf extract, the aqueous whole-plant extract, and the ethanolic leaf extract (Fig 2). Similar chemicals with analogous biological activity were also discovered in root aqueous, ethanolic aqueous, and whole plant extracts (Fig 2).

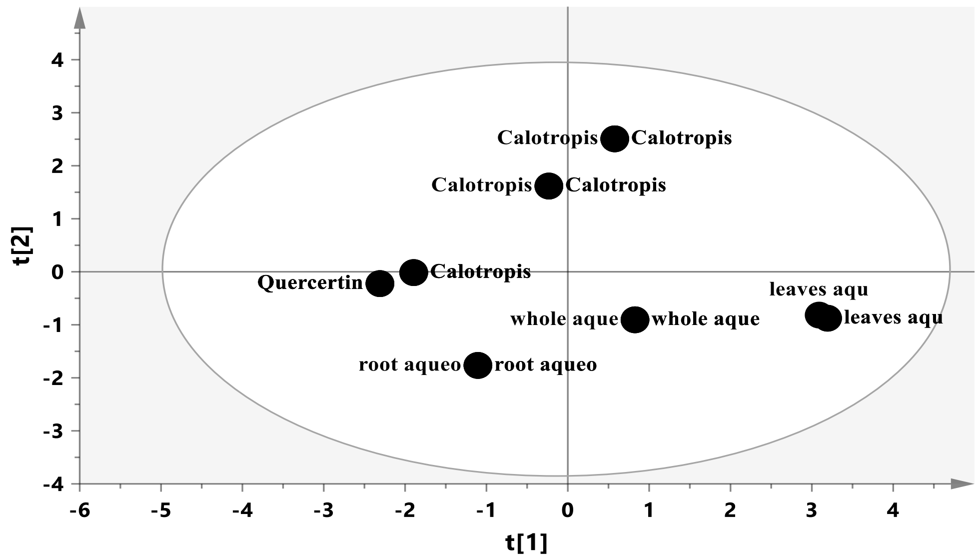

Fig. 2: PCA loading presenting the biological qualitative relationship of different parts of *C. procera*

Conclusions: The current study documented a variety of invaluable information regarding *C. procera*, a traditional medical knowledge in Kaduna state, northern Nigeria. Traditional remedies rely heavily on medicinal herbs. At 86.01 µg ML^{-1} and 18.1 mmol g^{-1}, the leaves showed the highest levels of radical scavenging, ferric reducing power respectively, and highest inhibition of 23.5 mm against *P. aeruginosa*. The results of the biological activity support the respondents' argument. The findings revealed the leaves of *C. procera* as a valuable resource to fight infectious diseases and enhance general health.

REFERENCES

Abdulrahman, M. 2021. Antioxidant, alpha glucosidase and antibacterial evaluation of *Syzygium mytifolium* (Roxb.) Walp. *Plant Science Today,* **8**(2): 410–415.

Abdulrahman, M.D., Ali, A., Fatihah, H., Khandaker, M. and Mat, N. 2019. *In vitro* biological investigations on *Syzygium polyanthum* cultivars. *International Journal of Agriculture and Biology,* **22**(6): 1399-1406.

Abdulrahman, M.D., Ali, A.M., Fatihah, H., Khandaker, M.M. and Mat, N. 2018. Traditional medicinal knowledge of Malays in Terengganu, Peninsular Malaysia. *Malayan Nature Journal,* **70**(3): 349-364.

Abdulrahman, M.D., Bradosty, S.W., Hamad, S.W., Ibrahim, M. T., Lema, A.A., Sunusi, N. and Wada, N. 2022. Traditional Methods for treatment and management of measles in Northern Nigeria: Medicinal plants and their molecular docking. *Ethnobotany Research and Applications,* **23**: 1-18.

Abo, K., Fred-Jaiyesimi, A. and Jaiyesimi, A. 2008. Ethnobotanical studies of medicinal plants used in the management of diabetes mellitus in South Western Nigeria. *Journal of Ethnopharmacology,* **11I5**(1): 67-71.

Dogara, A., Labaran, I., Hamad, S.W., Lema, A.A. and Jakada, B.H. 2021. Traditional medicinal plants used for the treatment of cancer in Mubi, Adamawa State, Nigeria. *Al-Qadisiyah Journal of Pure Science,* **26**(4): 258-268.

Dudonne, S., Vitrac, X., Coutiere, P., Woillez, M. and Mérillon, J.M. 2009. Comparative study of antioxidant properties and total phenolic content of 30 plant extracts of industrial interest using DPPH, ABTS, FRAP, SOD, and ORAC assays. *Journal of Agricultural and Food Chemistry,* **57**(5): 1768-1774.

Ijaz, F., Iqbal, Z., Rahman, I.U., Alam, J., Khan, S.M., Shah, G.M., Afzal, A. 2016. Investigation of traditional medicinal floral knowledge of Sarban hills, Abbottabad, KP, Pakistan. *Journal of Ethnopharmacology,* **179**: 208-233.

Jamalova, D.N., Gad, H.A., Akramov, D.K., Tojibaev, K.S., Musayeib, N.M.A., Ashour, M.L. and Mamadalieva, N.Z. 2021. Discrimination of the essential oils obtained from four apiaceae species using multivariate analysis based on the chemical compositions and their biological activity. *Plants,* **10**(8): 1529. [*10.3390/plants10081529*].

Jeruto, P., Lukhoba, C., Ouma, G., Otieno, D. and Mutai, C. 2008. An ethnobotanical study of medicinal plants used by the Nandi people in Kenya. *Journal of Ethnopharmacology,* **116**(2): 370-376.

Kankara, S.S., Nuhu, A.I., Bindawa, K.A., Haruna, M.R.U., Bello, A. and Abubakar, I.B. 2022. Indigenous traditional knowledge of medicinal plants used for the management of hiv/aids opportunistic infections in Katsina state, Nigeria. *Ethnobotany Research & Applications* **23**: 1-17.

Kayfi, S. and Abdulrahman, M.D. 2021. Ethnopharmacology of plants in choman, the kurdistan region of Iraq. *Applied Biological Research,* **23**(4): 322-330.

Kinda, P.T., Nacoulma, A.P., Guenné, S., Compaoré, M., Djandé, A., Lagnika, L. and Kiendrébéogo, M. 2020. The metabolomic study of *Calotropis procera* Ait. from Burkina Faso, based on chemical functional groups profiling using FTIR. *Journal of Complementary and Integrative Medicine,* **17**(3): 10.1515/jcim-2019-0134.

Kumar, V.L., Verma, S. and Das, P. 2022. Anti-inflammatory and antioxidant effect of methanol extract of latex of *Calotropis procera* in rat model of colorectal cancer. *Journal of Ethnopharmacology*, **296**: 115503. [*10.1016/j.jep.2022.115503*].

Mahmoud, A.D., Fatihah, H.N.N., Khandaker, M.M., Ali, A.M. and Mat, N. 2020. Ethnobotany of *Syzygium polyanthum* (Wight) Walp in Terengganu, Peninsular Malaysia. *Journal Of Agrobiotechnology*, **11**(2): 39-47.

Merouane, A., Fellag, S., Touaibia, M. and Beldi, A. 2022. A ethnobotanical survey of medicinal plants consumed during holy month of Ramadan in Chlef region, Algeria. *Ethnobotany Research and Applications,* **23**: 1-14.

Rani, R., Sharma, D., Chaturvedi, M. and Yadav, J.P. 2019. Phytochemical analysis, antibacterial and antioxidant activity of *Calotropis procera* and *Calotropis gigantea*. *The Natural Products Journal*, **9**(1): 47-60.

Sultana, B., Anwar, F. and Ashraf, M. 2009. Effect of extraction solvent/technique on the antioxidant activity of selected medicinal plant extracts. *Molecules,* **14**(6): 2167-2180.

Umarkar, A.R., Bavaskar, S.R., Bagad, Y.M., Barhate, S.D., Jadhav, R. and Makwana, A. 2011. *Calotropis procera* (AIT): A phytopharmacological review. *Research Journal of Pharmacognosy and Phytochemistry,* **3**(6): 256-260.

Yunusa, A.K., Rashid, Z.M., Mat, N., Bakar, C.A.A. and Ali, A.M. 2018. Chemicals and bioactivity discrimination of syconia of seven varieties of ficus deltoidea jack via atr-ir spectroscopic-based metabolomics. *Pharmacog Journal* **10**(6): 147-151.

Yunusa, A.K, Dandago, M.A., Ibrahim S.A.M, Abdullahi, N., Rilwan, A. and Barde A. 2018. Total phenolic content and antioxidant capacity of different parts of cucumber (*Cucumis sativus* L.). *Acta Universitatis Cinbinesis, Series E: Food Technology* **22**(2): 13-20.