World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 6.129

A STUDY ON CYTOTOXIC AND ANTIOXIDANT ACTIVITY OF *COMBRETUM DENSIFLORUM* EXTRACTS

Sushri Sangita Rout and Sunita Bhatnagar*

Medicinal and Aromatic Plants, Regional Plant Resource Centre, Ekamra Kannan, Bhubaneswar, India.

***Corresponding Author: Dr. Sunita Bhatnagar**
Medicinal and Aromatic Plants, Regional Plant Resource Centre, Ekamra Kannan, Bhubaneswar, India.

Article Received on 11/10/2023 **Article Revised on 01/11/2023** **Article Accepted on 21/11/2023**

ABSTRACT

Combretum densiflorum is a member of family Combretaceae grown for ornamental purpose. Solvent extracts of leaf and bark were explored for their cytotoxic and antioxidant activities. Cytotoxic activity was studied using brine shrimp assay, chloroform extract of leaf and acetone extract of bark revealed maximum activity to the tune of 98 to 100 percent suggesting the presence of some active principles in both the samples. All the other extracts showed mild to moderate activity but one common thing was the presence of dose dependent activity in all. Antioxidant activity was conducted using TLC based DPPH assay. As far as antioxidant activity is concerned a total of 13 antioxidant bands were revealed in bark samples, while 4 antioxidant bands were obtained in leaf extracts.

KEYWORD: *Combretum densiflorum*, Combretaceae, Cytotoxic activity, antioxidant activity.

INTRODUCTION

Combretum densiflorum is a cultivated ornamental plant belonging to family Combretaceae. It is chiefly confined to tropics and consists 20 genera with 600 species.[1] The plants of Combretaceae family constitute all sorts of flora such as trees, shrub and herbs.[2] Family is well known for a number of medicinal plants like *Terminalia arjuna, Terminalia bellerica, Terminalia chebula, Combretum pilosum, Combretum roxburghii etc.* The raw leaves *Combretum decandrum* are used in diarrohea, other gastric troubles, in skin-disease by khasi and garo tribes of Meghalaya.[3] The leaves of *Combretum pilosum* are anthelmintic and a decoction of the leaves is considered to be specific for *Ascaris lumbricoides* and *Oxyuris vermicularis.*[4] The leaves of *C. acuminatum* Roxb, and the fruits of *C. trifoliatum* Vent. are anthelmintic, the former being effective against tape worm and latter against Ascaris.[5] One of the most popular ayurvedic formulation is Triphala made from Three Terminalia species.[6] Thus, keeping in view of the significant use of members of Combretaceae family, it was thought worthwhile to explore this ornamental species i.e., *Combretum densiflorum* for its biological potential.

MATERIALS AND METHODS

Collection and Processing of plant material

The leaves and bark of medicinal plant *Combretum densiflorum* were collected from medicinal germplasm garden of Regional Plant Resource Centre, Bhubaneswar, India. Leaves and bark were washed thoroughly under running tap water to remove dust and then these were allowed to dry in shade at room temperature. After drying the leaves and bark were grinded properly by using Lexus grinder.

***Moisture content*:** Moisture content was calculated for bark as well as leaf sample by comparing the weight of dried leaves with that of fresh leaves.

Solvent extraction

Solvent extraction of leaf and bark samples was done on polarity basis i.e. from non-polar hexane to polar methanol by using Soxhlet Apparatus as per the standard protocols.[7] Four extracts namely hexane, chloroform, acetone, methanol were prepared using successive solvent extraction method respectively. These extracted samples were further concentrated by using Buchi (R-200) Rotavapour. The concentrated samples were stored in refrigerator for further use.

Biological evaluation

1. Brine shrimp lethality test

Brine shrimp (Artemia salina) eggs were incubated for 48hrs in 8% saline water to get the desired growth of the larvae for biological evaluation. Stock solution of different extracts was prepared at a concentration of 10mg/ml. Larvae were subjected to different concentrations of extracts and their motility was compared with that of controlled experimental tubes containing larvae without any drug concentration. Motility Readings were taken every hour up to 4hours. Motility was graded as below:

4+ highly motile
3+ motile
2+ sluggish
1+ slow
Nil no activity at all

After 24hrs the final reading was taken and percentage of inhibition was calculated[8] by counting the surviving larvae and comparing them with the number of surviving larvae in controls.

2. Antioxidant activity of combretum densiflorum extracts

To detect antioxidant activity, qualitative 2, 2 diphenyl-1-picrylhydrazyl (DPPH) assay was carried out. The plates were first air dried and then the chromatograms were sprayed with 0.2% 2, 2 diphenyl-1-picryl-hydrazyl in methanol as an indicator.[9] The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC plates sprayed with 0.2%DPPH in methanol.

Qualitative screening of the constituents in each of the leaf and bark crude extracts of *Combretum albidum* for antioxidant activity was done by TLC analysis. The process was carried out using TLC sheets. For about 5µl of each sample was loaded on the TLC sheet and the chromatograms were developed in following solvent systems:

- Ethyl acetate: Methanol : water (40:5.4:4) [EMW] (polar neutral);
- Chloroform: Ethylacetate : formic acid (5:4:1) [CEF] (intermediate polarity/acidic);
- Benzene: Ethanol: ammonium hydroxide (90:10:1) [BEA] (Nonpolar/basic) After development of chromatograms upto 8cms, they were taken out and sprayed with DPPH. Yellow bands were counted as mentioned above.

RESULTS AND DISCUSSION

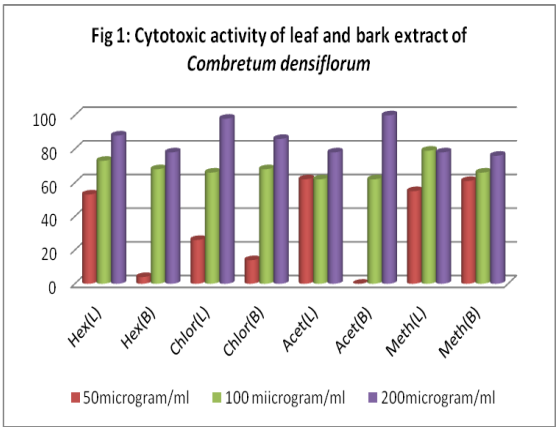
As expected moisture content of leaves was more than the bark at 69 and 50% respectively. As can be observed from Table 1, yield of methanol extract was highest in leaf and bark however yield of leaf extracts was more in comparison to the bark extracts. Results clearly indicate that polar molecules in both the samples were abundant when compared with non polar extracts.

Table 1: Yield of solvent extracts of leaf and bark samples of *Combretum densiflorum*.

S. no.	Solvent extracts	Yield (%) Leaves	Yield (%) Bark
1	Hexane	2.6	0.33
2	Chloroform	2.8	1.31
3	Acetone	1.7	0.63
4	Methanol	17.3	8.81

Cytotoxic activity using brine shrimp lethality assay

As can be seen from Fig 1, majority of the solvent extracts showed dose dependent activity against brine shrimp assay. Maximum activity was obtained in chloroform leaf extract and acetone bark extract at a higher dose of 200 microgram/ml. As activity in both the extracts is almost 100% so both the extracts are potent candidates for exploration of cytotoxic activity in other test models.



Antioxidant activity of Combretum densiflorum Leaf and bark extracts

As observed from the tables 2 a number of antioxidant molecules with different Rf values were obtained in bark as well as leaf samples. Amongst bark sample acetone extract was the one with maximum number of antioxidant bands as shown in below. However acetone extract of leaf showed only two bands in EMW solvent. A band at Rf of 0.68 was common in acetone extract of bark as well as leaf in the above solvent.

Leaf methanol extract consisted of a large number of molecules situated very closely so streak was obtained instead of bands. As per the antioxidant activity was concerned acetone extracts showed large number of clear bands.

Table 2: Antioxidant activity of leaf and bark extracts of *combretum densiflorum.*

Solvent system	Hexane extract(Rf)		Chloroform extract(Rf)		Acetone extract(Rf)		Methanol extract(Rf)	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Ethyl acetate: methanol: water (40:5.4:4) [EMW]	NIL	NIL	NIL	NIL	0.53, 0.68	0.06,0.16, 0.68,0.75, 0.81	Streak	0.06, 0.15, 0.75,
Chloroform: ethylacetate: formic acid (5:4:1) [CEF]	NIL	NIL	NIL	NIL	0.12	NIL	Streak	NIL
Benzene: ethanol: ammonium hydroxide (90:10:1) [BEA]	0.93	NIL	NIL	0.46	NIL	0.06,0.15, 0.46	NIL	0.06

As plant was rich in cytotoxic as well as antioxidant activity hence can be considered at par with other members of the family Combretaceae.

ACKNOWLEDGEMENTS

Authors acknowledge the Forest, Environment and Climate change Department, Government of Odisha for providing funding for the smooth conduct of entire work and also acknowledge Chief Executive, Regional Plant Resource Center, Bhubaneswar, Odisha for providing lab facilities.

REFERENCES

1. de Morais Lima GR, de Sales IR, Caldas Filho MR, de Jesus NZ, de Sousa Falcão H, Barbosa-Filho JM, Cabral AG, Souto AL, Tavares JF, Batista LM. Bioactivities of the genus Combretum (Combretaceae): a review. Molecules, 2012; 17(8): 9142-206. doi: 10.3390/molecules17089142.
2. Parusnath M, Naidoo Y, Singh M, Rihan H, Dewir YH. Phytochemical Composition of *Combretum molle* (R. Br. ex G. Don.) Engl. & Diels Leaf and Stem Extracts. Plants (Basel), 2023; 12(8): 1702. doi: 10.3390/plants12081702.
3. Toklo PM, Yayi Ladekan E, Linden A, Hounzangbe-Adote S, Kouam SF, Gbenou JD. Anthelmintic flavonoids and other compounds from *Combretum glutinosum* Perr. ex DC (Combretaceae) leaves. Acta Crystallogr C Struct Chem, 2021; 77(9): 505-512. doi: 10.1107/S2053229621007841.
4. Dawé A, Mbiantcha M, Yakai F, Jabeen A, Ali MS, Lateef M, Ngadjui BT. Flavonoids and triterpenes from *Combretum fragrans* with anti-inflammatory, antioxidant and antidiabetic potential. Z Naturforsch C J Biosci, 2018; 73(5-6): 211-219. doi: 10.1515/znc-2017-0166.
5. Ramsay GC. *Combretum pilosum* Roxb. as an Anthelmintic for Ascaris Lumbricoides. Ind Med Gaz, 1922; 57(10): 374-375.
6. Peterson CT, Denniston K, Chopra D. Therapeutic Uses of Triphala in Ayurvedic Medicine. J Altern Complement Med, 2017; 23(8): 607-614. doi: 10.1089/acm.2017.0083.
7. Behera DR, Bhatnagar S. Assessment of macrofilaricidal activity of leaf extracts of Terminalia sp. against bovine filarial parasite Setaria cervi. J Infect Public Health, 2018; 11(5): 643-647. doi: 10.1016/j.jiph.2018.01.006.
8. D'Souza P, Deepak M, Rani P, Kadamboor S, Mathew A, Chandrashekar AP, Agarwal A. Brine shrimp lethality assay of Bacopa monnieri. Phytother Res, 2002; 16(2): 197-8. doi: 10.1002/ptr.1001.
9. Gurgel RS, de Melo Pereira DÍ, Garcia AVF, Fernandes de Souza AT, Mendes da Silva T, de Andrade CP, Lima da Silva W, Nunez CV, Fantin C, de Lima Procópio RE, Albuquerque PM. Antimicrobial and Antioxidant Activities of Endophytic Fungi Associated with *Arrabidaea chica* (Bignoniaceae). J Fungi (Basel), 2023; 9(8): 864. doi: 10.3390/jof9080864.