**ABSTRACT**

**Aims:** The aim of this study is to investigate the carbonic anhydrase inhibitory potential of *Cadaba farinosa* leaf extract.

**Methodology:** *Cadaba farinosa* leaves were extracted using soxhlet apparatus by *n*-Hexane, followed by Ethyl acetate and then Methanol. The extracts were tested against *in vitro* carbonic anhydrase activity. The most active extract was further purified to determine *in vitro* the most active fraction against carbonic anhydrase activity. The purified fractions of Methanol extracts were subjected to FTIR and GC-MS analysis to determine the functional compounds responsible of such biological activity.

**Results:** The result of the present study revealed the presence of inhibitory activity of *n*-Hexane and
Ethyl acetate extract against carbonic anhydrase whereas the Methanol extract showed activation against the enzyme. Purified fractions of Methanol leaf extract revealed the presence of activator and inhibitor fraction. FTIR analysis of the inhibitor fraction of methanol leaf extract revealed characteristic band absorption similar to alpha aminonitrile while the GC-MS analysis revealed the presence of derivatives ribonitrile, of methyl hydrazine, methyl pyridine.

**Conclusion:** *Cadaba farinosa* leaf has compounds that can inhibit and activate carbonic anhydrase, and thus could be used in the development of nitrile-containing pharmaceuticals which are prescribed for a diverse variety of medicinal indications.

**Keywords:** *Cadaba farinosa; carbonic anhydrase; aminonitrile; hydrazine.*

### 1. INTRODUCTION

Many natural and synthetic substances can affect the living metabolism by altering enzyme activities and affecting metabolic pathways at low concentrations [1,2]. Many chemicals at relatively low dosages affect the metabolism of organisms by altering normal enzyme activity, particularly inhibition of a specific enzyme [3]. The effects can be dramatic and systemic [4]. Carbonic anhydrase, is a well-characterized pH-regulatory metalloenzyme, that rapidly catalyzes the hydration of carbon dioxide (CO$_2$) to form bicarbonate (HCO$_3^-$) and the reversible dehydration [5,6]. The conversion of carbon dioxide and water to carbonic acid by carbonic anhydrase contributes to the acidification of the extracellular microenvironment [7,8,9]. The acidic extracellular environment associated with tumors in turn promotes the expression of proteinases that contribute to invasion and metastasis [10,11]. Compounds capable of inhibiting carbonic anhydrase activity and therefore reducing carbonic acid formation via increased carbonic anhydrase activity associated with many cancers is a strategy that can be employed to repress invasion and metastasis of cancer cells.

Inhibition of some Carbonic anhydrase isoforms by aromatic/heterocyclic compounds has been exploited in therapy for many years. Such pharmacological agents were shown to be useful as diuretics, or in the treatment and prevention of a variety of diseases such as glaucoma, epilepsy, congestive heart failure, mountain sickness, gastric and duodenal ulcers, neurological disorders and osteoporosis, among others [12,13,14]. However, as carbonic anhydrases are ubiquitous enzymes in vertebrates, administration of systemic inhibitors leads to CA inhibition in organs other than the target (i.e., the eye), and to undesired side effects as a result of these drugs. The most frequent ones are: numbness and tingling of extremities; metallic taste; depression; fatigue; malaise; weight loss; decreased libido; gastrointestinal irritation; metabolic acidosis; renal calculi and transient myopia [15,16,17]. Thus, it is essential to look for more effective carbonic anhydrase inhibitor agents with fewer side effects. *Cadaba farinosa* leaf is used by locals in some parts of Jigawa state of Nigeria for the treatment of cancer and diabetes. According to ethnomedical information the plant *Cadaba farinosa* has been used for treating various ailments such as inflammation and diabetes and in female fertility [18,19]. In view of these observations, the aim of this study was to investigate the in vitro inhibitory effects of *Cadaba farinosa* leaf extract on carbonic anhydrase activity, since changes of carbonic anhydrase activities have been reported to alter in many pathological conditions.

### 2. MATERIALS AND METHODS

#### 2.1 Experimental

All the chemicals and solvents used for the study were of reagent grade and were obtained commercially from Sigma Aldrich (England). Bovine erythrocyte carbonic anhydrase was purchased from Sigma Aldrich (England). P-nitrophenyl acetate and Trizma base were purchased from Sigma Aldrich (England). The infrared spectra of the purified fractions were recorded on a Thermo Scientific Nicolet iS10 (England) FTIR Spectrophotometer, assisted by a computer, in the solid state in the 4000–400 cm$^{-1}$. The reported wave-numbers are estimated to be accurate to within ± 3 cm$^{-1}$. The GC-MS analysis were performed using a GCMS-QP2010 Plus Shimadzu (Japan), Hewlett Packard gas chromatograph (model 6890) equipped with a flame ionization detector and injector MS transfer line temperature of 230°C respectively. Analyses was carried out on a Agilent Technologies Network mass spectrometer (model 5973) coupled to H.P. gas chromatograph (model 6890) equipped with NBS 75K Library Software
database. Mass spectra was recorded at 70 eV /200°C.

2.2 Plant Material

_Cadaba farinosa_ leaves were collected from ringim local government area, of Jigawa state and authenticated at the Biological science Department of Ahmadu Bello University Zaria, Nigeria. A voucher number was given as no. 2744 and was deposited at the herbarium of the department. The leaves were, washed with distilled, air-dried at room temperature for 72 hours, grinded to powder.

2.3 Extraction and Purification

Leaves of _Cadaba farinosa_ were dried in shade at room temperature and grounded to powder using pestle and mortar. The powder was extracted with n-Hexane, followed by Ethyl acetate and methanol using successive soxhlet-extraction for a period of 72 hours each. The extract was filtered through a Buchner funnel with Whatman filter paper No.1. The filtrate was evaporated to dryness under reduced pressure using rotary evaporator. The methanol extract after complete evaporation of the solvent, was subjected to column chromatography to perform purification of the extract. Fractions with similar TLC profiles were pooled together and analyzed to ascertain the purification purity.

2.4 Carbonic Anhydrase Assay

The activities of the fractions were determined as mentioned by [20], with the modification described by [21], by the release of p-nitrophenol by hydrolysis of the synthetic substrate, p-nitrophenyl acetate at room temperature by carbonic anhydrase enzyme. The assay was conducted in a total of 3 mL reaction volume. For inhibition assay, a fraction (15 µg/100 µL in DMSO) was mixed with 10 µg/100µL enzyme for about an hour at room temperature before reaction with the substrate (1.4 ml 0.05 M Tris-HCl buffer, pH: 7.4 and 1.5 ml p-nitrophenyl acetate). The reaction was initiated by the addition of the enzyme mixture with the fraction, after zeroing with the substrate (p-nitrophenyl acetate (pNPA, 3 mM)). The release of p-nitrophenol was determined by measuring the absorbance at 345 nm after monitoring continuously for 3 min. One unit of enzyme activity was expressed as 1 µmol of released p-nitrophenol per minute at room temperature.

3. RESULTS

3.1 In vitro Effect of Crude n-Hexane, Ethyl Acetate and Methanol Extracts of _Cadaba farinosa_ on Carbonic Anhydrase Activity

Figs. 1a and b shows the effect of crude n-Hexane, ethyl acetate and Methanol extracts of _Cadaba farinosa_ on carbonic anhydrase activity. Among the three crude extract of _Cadaba farinosa_ leaves, methanol extract has produced increase of carbonic anhydrase activity with 9.0% activation, while n-Hexane and ethyl acetate extract produced a significant reduction in carbonic anhydrase activity with 65.9% and 72.4% inhibition.

![Fig. 1a. In vitro inhibitory effect of Acetazolamide, Metformin and leaf extract of _Cadaba farinosa_ on bovine erythrocyte carbonic anhydrase activity treated at 15 µg/10 µg control concentration each](image-url)

---

Ibrahim et al.; IJBCRR, 11(4): 1-8, 2016; Article no.IJBCRR.24604
Fig. 1b. Percentage inhibition of Acetazolamide, Metformin and leaf extract of Cadaba farinosa on Bovine erythrocyte carbonic anhydrase activity treated at 15 µg/10 µg control concentration each

3.2 In vitro Effect of Purified Fraction of Methanol Leaf Extract of Cadaba farinosa on Carbonic Anhydrase Activity

Only fraction A showed higher (44.1%) inhibition when compared with fraction B (13.2%), C (5.3%) and D (7.9%), respectively. However, only fraction E showed increased carbonic anhydrase activity (Figs. 2a and b).

3.3 Infrared Spectra Analysis

The infrared spectra of Purified fraction A of Cadaba farinosa leaf extract showed a single band at 3486.91 cm\(^{-1}\) which was assigned to symmetric vibration of NH\(_2\) group. The corresponding NH\(_2\) appeared in the 3400-3500 cm\(^{-1}\) region characteristic band of Amine group present in the spectrum. A single band at 2226.31 cm\(^{-1}\) region is tentatively assigned to Nitrile group (C\(\equiv\)N) which appeared in the 2220-2240 cm\(^{-1}\) region. The infrared spectral data of fraction A in (Table 1a) showed some characteristic bands related to \(\alpha\)-aminonitriles; which could be attributed to C\(\equiv\)N group. Finally bands at 2932.73, 1719.05 and 1653.86 cm\(^{-1}\) were assigned to Alkane, Ketone and Alkene respectively (Table 1a).

However, the infrared spectra of Purified fraction E of Cadaba farinosa leaf extract also showed a single band at 3398.37 cm\(^{-1}\) which was assigned to symmetric vibration of NH\(_2\) group (Table 1b). The corresponding NH\(_2\) appeared in the 3400-3500 cm\(^{-1}\), but it does not show any band within 2220-2240 cm\(^{-1}\) region, a characteristic region of Nitrile group. A band at 2921.74 and 1743.64 cm\(^{-1}\) were assigned to Alkane and esters respectively.

Fig. 2a. In vitro inhibitory effect of Acetazolamide, Metformin and purified fractions of methanol leaf extract of Cadaba farinosa on bovine erythrocyte carbonic anhydrase activity treated at 15 µg/10 µg enzyme concentration each
Ibrahim et al.; IJBCRR, 11(4): 1-8, 2016; Article no.IJBCRR.24604

Fig. 2b. Percentage inhibition of Acetazolamide, Metformin and purified fractions of methanol leaf extract of *Cadaba farinosa* on bovine erythrocyte carbonic anhydrase activity treated at 15 µg/10 µg enzyme concentration each

Table 1a. Functional groups identified using FTIR spectra analysis for the inhibitor (fraction A) of methanol leaf extract of *Cadaba farinosa*. Wavenumbers (cm⁻¹)

|   | Absorbance | Absorption ranges | Functional group names |
|---|------------|-------------------|-----------------------|
| 1 | 3486.91    | 3400-3500         | N-H, AMINE            |
| 2 | 2932.73    | 2850-3000         | C-H, ALKANE (stretch) |
| 3 | 2226.31    | 2220-2240         | CN, NITRILE           |
| 4 | 1719.05    | 1710-1720         | >C=O, KETONE          |
| 5 | 1653.86    | 1640-1670         | C=C, ALKENE           |

Table 1b. Functional groups identified using FTIR spectra analysis for the activator (fraction E) of methanol leaf extract of *Cadaba farinosa*. Wavenumbers (cm⁻¹)

|   | Absorbance | Absorption ranges | Functional group names               |
|---|------------|-------------------|--------------------------------------|
| 1 | 3398.37    | 3400-3500         | N-H, AMINE                           |
| 2 | 2921.74    | 2850-3000         | C-H, ALKANE                          |
| 3 | 1743.64    | 1735-1750         | R-C(O)-O-R, ESTERS (aliphatic)       |
| 4 | 1456.59    | 1450-1470         | C-H, ALKANE (bend)                   |

3.4 Gas Chromatography-mass Spectra Analysis

The GC-MS result of the purified fraction A revealed the presence of 2,3,4,5-tetraacetate-D-Ribonitrile with the molecular formula C₁₃H₁₇NO₈, 4-methyl-3-(2-methylhydrazino)-Phenol with molecular formula C₉H₁₂N₂O, 5-Ethyl-2-methylpyridine 1-oxide with molecular formula C₈H₁₁NO, 1,2-Dimethyl-3-nitro-4-nitroso-benzene with molecular formula C₉H₈N₂O₃ and Octanamide with molecular formula C₁₈H₃₇NO (Table 2a). Whereas fraction E of the purified extract revealed the presence of Hexadecanoic acid, 1-[[[[2-aminoethoxy]hydroxyphosphinyl]oxy[methyl]-1,2-ethanediyl ester with molecular formula C₃₇H₇₄NO₈P, 9-Octadecenamide with molecular formula C₁₈H₃₅NO, n-Decyl fluoride With molecular weight formula C₁₈H₂₁F, Eicosanoic acid with molecular formula C₂₀H₄₀O₂ and Ethylene glycol monooleate With molecular formula C₂₀H₃₈O₃ (Table 2b).
Table 2a. Compounds identified using GC-MS analysis from the inhibitor (fraction A) of methanol leaf extract fraction of *Cadaba farinosa*. Wavenumbers (cm\(^{-1}\))

| S/N | Compound name | Peak | R. time | Formula | M. Wt. |
|-----|---------------|------|---------|---------|--------|
| 1   | N-Ethoxyisobuten-3-imine | 1 | 4.950 | C\(_6\)H\(_{11}\)NO | 113 |
| 2   | 2,3,4,5-tetraacetate-D-Ribonitrile, | 2 | 8.833 | C\(_{13}\)H\(_{17}\)NO\(_8\) | 315 |
| 3   | 4-methyl-3-(2-methylhydrazino)-Phenol | 79 | 11.767 | C\(_8\)H\(_{12}\)N\(_2\)O | 152 |
| 4   | 5-Ethyl-2-methylpyridine 1-oxide | 79 | 11.767 | C\(_8\)H\(_{11}\)NO | 137 |
| 5   | 1,2-Dimethyl-3-nitro-4-nitroso-benzene | 3 | 11.767 | C\(_8\)H\(_8\)N\(_2\)O\(_3\) | 180 |
| 6   | 9-Octadecenamide | 15 | 23.167 | C\(_{18}\)H\(_{36}\)NO | 281 |

Table 2b. Compounds identified using GC-MS analysis from the activator (fraction E) of methanol leaf extract fraction of *Cadaba farinosa*. Wavenumbers (cm\(^{-1}\))

| S/N | Compound name | Peak | R. time | Formula | M. Wt. |
|-----|---------------|------|---------|---------|--------|
| 1   | Hexadecanoic acid, 1-[[[(2-aminoethoxy)hydroxyphosphinyl]oxy]methyl]-1,2-ethanediyl ester | 8 | 22.392 | C\(_{27}\)H\(_{47}\)NO\(_8\)P | 691 |
| 2   | 9-Octadecenamide | 9 | 23.125 | C\(_{18}\)H\(_{36}\)NO | 281 |
| 3   | n-Decyl fluoride | 8 | 23.392 | C\(_{10}\)H\(_{21}\)F | 160 |
| 4   | Eicosanoic acid | 7 | 20.992 | C\(_{20}\)H\(_{40}\)O\(_2\) | 312 |
| 5   | Ethylene glycol monooleate | 13 | 26.225 | C\(_{20}\)H\(_{38}\)O\(_3\) | 326 |

4. DISCUSSION

*In vitro* study of the purified Methanol fraction, showed fraction A inhibiting carbonic anhydrase activity while fraction E activating carbonic anhydrase activity, the difference was seen in the FTIR spectra data showing the presence of aminonitrile containing groups in the fraction A and none in the fraction E, suggesting the presence of the nitrile containing group may be responsible for the inhibitory activity of the inhibitor fraction A. GC-MS analysis of fraction A further revealed the presence of some compounds which are: Ribonitrile, methyl hydrazine, methyl pyridine and octanamide. Hydrazine derivatives are reported to be environmental and food pollutants but are also important because of their use in medicine for the treatment of tuberculosis and cancer, and are also reported to pose significant health risks to humans as they are mutagenic and carcinogenic [22]. Hydrazine and its derivatives, which are used as high energy rocket fuel, induce a variety of toxic insults, including hypoglycemia, disorders of the CNS, induction of systemic lupus erythematosus, and cancer [23-25]. Hydrazines are also found in tobacco and in edible mushrooms, and in Isoniazid and iproniazid, monoamine oxidase inhibitors, are in use for the treatment of tuberculosis and, until recently, as an antidepressant, respectively [26,27]. Leaf extract of *Cadaba farinosa* is being used for the treatment of cancer and diabetes in parts of Jigawa state of Nigeria as folk medicine. In the current study, we demonstrated for the first time that *Cadaba farinosa* methanol leaf extract has amino-nitrile containing compounds from the FTIR spectra analysis, which showed absorption characteristic bands related to \(\alpha\)-aminonitriles at 2226.31 cm\(^{-1}\) region which is tentatively assigned to Nitrile group (C≡N). Over 30 nitrile-containing pharmaceuticals are prescribed for a diverse variety of medicinal indications with more than 20 additional nitrile-containing leads in clinical development. Several amino nitriles have been developed as reversible inhibitors of dipeptidyl peptidase (DPP IV) for treating diabetes [28]. Vildagliptin is a recently released aminonitrile-containing antidiabetic drug that inhibits dipeptidyl peptidase IV (DPP-IV).

Several nitrile-containing drugs are in use for a variety of medical treatments. Such as the blockbuster drug Anastrazole, marketed by AstraZeneca under the trade name Arimidex, is considered the drug of choice for treating oestrogen-dependent breast cancer, etravirine is a nitrile containing anti HIV agent, while (rilpivirine) are among the many etravirine analogs under development, which is being touted as among “the most potent anti-HIV agent(s) ever discovered.” [29] and Entacapone used for treating Parkinson’s disease is a potent inhibitor of catecholamine-O-methyltransferase. And numerous candidates are currently being pursued in clinical trials [30].
5. CONCLUSION

We may conclude that the use of leaf extract of *Cadaba farinosa* by locals for the treatment particularly of cancer and diabetes, may be justified by the presence of characteristic band absorption of alpha aminonitrile functional component, and also the presence of hydrazine derivative which may explain the anti-cancer activity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Beydemir S, Çiftçi M, Ozmen I, Büyükokuru_Iu ME, Özdemir H, Kürfrevioğlu OI. Effects of some medical drugs on enzyme activities of carbonic anhydrase from human erythrocytes in vitro and from rat erythrocytes *In vivo*. Pharmacol. Res. 2000;42:188-191.

2. Gidaro MC, Alcaro F, Carradori S, Costa G, Vullo D, Supuran CT, Alcaro S. Eriocitrin and apigenin as new carbonic anhydrase VA inhibitors, from a virtual screening of Calabrian natural products. Planta Medica. 2015;81:533-540.

3. Christensen GM, Olson D, Riedel B. Chemical effects on the activity of eight enzymes: A review and a discussion relevant to environmental monitoring. Environ. Res. 1982;29:247-255.

4. Hochster RM, Kates M, Quastel JM. Metabolic Inhibitors, Academic Press, New York. 1972;3:4-71-89.

5. Gambhir KK, Oates P, Verma M, Temam S, Cheatham W. High fructose feeding enhances erythrocyte carbonic anhydrase 1 mRNA levels in rat. Ann. N. Y. Acad. Sci. 1997;827:163-169.

6. Kendall AG, Tashian RE. Erythrocyte carbonic anhydrase I: Inherited deficiency in humans. Science. 1977;197:471-472. DOI: 10.1126/science.406674

7. Supuran CT, Scozzafava A. Carbonic anhydrases as targets for medicinal chemistry. Bioorganic and Medicinal Chemistry. 2007;15:4336-4350.

8. Fukumura D, Jain RK. Tumor microenvironment abnormalities: Causes, consequences, and strategies to normalize. J Cell Biochem. 2007;101:937-949.

9. Pastorekova S, Ratcliffe PJ, Pastorek J. Molecular mechanisms of carbonic anhydrase IX-mediated pH regulation under hypoxia. BJU Int. 2008; 101(Suppl 4):8-15.

10. Rofstad EK, Mathiesen B, Kindem K, Galappathi K. Acidic extracellular pH promotes experimental metastasis of human melanoma cells in athymic nude mice. Cancer Res. 2006;66:6699-6707.

11. Stubbs M, McSheehy PJ, Griffiths JR, Bashford CL. Causes and consequences of tumour acidity and implications for treatment. Molecular Medicine Today. 2000;6:15-19.

12. Scozzafava A, Mastrolorenzo A, Supuran CT. Carbonic anhydrase inhibitors and activators and their use in therapy. Expert Opin. Ther. Pat. 2006;16:1627-1664.

13. Supuran CT, Scozzafava A, Conway J. Carbonic Anhydrase — Its inhibitors and Activators 1–363 (CRC, Boca Raton); 2004.

14. Carradori S, Mollica A, De Monte C, Granese A, Supuran CT. Nitric oxide donors and selective carbonic anhydrase inhibitors: A dual pharmacological approach for the treatment of glaucoma, cancer and osteoporosis. Molecules. 2015;20:5667-5679.

15. Bartlett JD, Jaanus SD. Clinical ocular pharmacology. Butterworth, Boston. 1989:254-263.

16. Maren TH: Role of carbonic anhydrase in aqueous humor and cerebrospinal fluid formation. In: Barriers and Fluids of the Eye and Brain. Segal, MB (Ed.), MacMillan Press, London. 1992;37-48.

17. Maren TH. The development of topical carbonic anhydrase inhibitors. J. Glaucoma.1995;4:49-62.

18. Tenpe CR, Upaganlawar AB, Yeole PG. Antidiabetic activity of *Cadaba indica* in Alloxan induced diabetic rats. Ind J. Nat. Prod. 2006;22(2):14-17.

19. Nadkarni AK. Indian Materia Medica. 3 rd ed. Bombay. Popular Prakashan. 2002;(I): 225-226.

20. Verpoorte JA, Mehta S, Edsall JT. Esterase activities of human carbonic anhydrase. J. Biol. Chem. 1967;242:18: 4221-4229.

21. Parui R, Gambir KK, Mehrotra PP. Changes in carbonic anhydrase may be the initial step of altered metabolism in hypertension. Biochem Int. 1991;23:779–89.
22. Sinha BK, Mason RP. Biotransformation of hydrazine derivatives in the mechanism of toxicity. J Drug Metab Toxicol. 2014;5:3: 2-6.

23. Trohalaki S, Zellmer RJ, Pachter R, Hussain SM, Frazier JM. Risk assessment of high-energy chemicals by In vitro toxicity screening and quantitative structure-activity relationships. Toxicol Sci. 2002;68: 498-507.

24. Fortney SR. Effect of hydrazine on liver glycogen, arterial glucose, lactate, pyruvate and acid-base balance in the anesthetized dog. J Pharmacol Exp Ther. 1966;153:562-568.

25. Toth B. Tumorigenic effect of 1-hydrazinophthalazine hydrochloride in mice. J Natl Cancer Inst. 1978;61:1363-1365.

26. Mitchell JR, Zimmerman HJ, Ishak KG, Thorgeirsson UP, Timbrell JA, et al. Isoniazid liver injury: Clinical spectrum, pathology, and probable pathogenesis. Ann Intern Med. 1976;84:181-192.

27. Nelson SD, Mitchell JR, Timbrell JA, Snodgrass WR, Corcoran GB. 3rd Isoniazid and iproniazid: Activation of metabolites to toxic intermediates in man and rat. Science. 1976;193:901-903.

28. Khun B, Hennig M, Mattei P. Molecular recognition of ligands in dipeptidyl peptidase IV. Curr. Top. Med. Chem. 2007;7:609-619.

29. De Clercq E. Emerging anti-HIV drugs. Expert Opin. Emerging Drugs. 2005; 10:241-274.

30. Costante R, Stefanucci A, Carradori S, Novellino E, Mollica A. DPP-4 inhibitors: A patent review (2012-2014). Expert Opin. Ther. Pat. 2015;25:209-236.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/14140