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RESEARCH ARTICLE

In Vitro Anti-cancer, Anti-hypertensive and Anti-hyperglycaemic effect of *Hypoxis colchicifolia*

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Abstract

Economic challenges associated with non-communicable diseases and the sociocultural outlook of many patients especially in Africa has increased the dependence on traditional herbal medicines for these diseases. *Hypoxis colchicifolia* is a traditional medicinal plant used in Southern Africa against an array of ailments. This study evaluated the *in vitro* anti-diabetic (α-amyalse and α-glucosidase), antihypertensive (angiotensin-converting enzyme) and anticancer potential of *H. colchicifolia* corm as well as leaf (acetone, methanol and aqueous) extracts. Results showed that extracts have a moderate anti-diabetic and anti-hypertensive potential, with great anti-cancer potential. The acetone extract of both fresh and dried corms produced significant α-amylase and α-glucosidase inhibition with ACE inhibited predominantly by the dried corms methanolic extract (IC_{50} 368.2 µg/mL). Methanolic extract of dried leaves showed the least cytotoxicity against the non-cancerous cell line HEK-293 while exhibiting the highest inhibition of MCF-7 cells (IC_{50} 3.24 µg/mL). All extracts exhibited a greater inhibitory potential in A549 cells than the positive control camptothecin (IC_{50} 304.2µg/mL). This study reveals that *H. colchicifolia* has therapeutic potential as an anti-diabetic and anticancer agent; however, further in vivo studies need to be conducted.

Keywords: Anti-diabetic, Antihypertensive, Anticancer, *Hypoxis colchicifolia*

Highlights

- *Hypoxis colchicifolia* is one of the four most selected plant species in traditional medicine, however their efficacy still needs to be scientifically validated.
- The distinct plant parts have different activities and cannot be used interchangeably.
- *Hypoxis colchicifolia* leaves has potential as both an anti-diabetic and anticancer agent.
- The dried corm methanolic extract shows effective activity as an anti-hypertensive agent.

1. Introduction

Non-Communicable Diseases (NCD) are the leading cause of death globally, with the top killers that together accounts for more than 80% of all precipitate NCD deaths including hypertension (17.9 million deaths annually), cancer (9.0 million) and diabetes (1.6 million)[1]. Similarly, in South Africa, diabetes, cancer and hypertension remain the greatest cause of morbidity. Conventional treatment for each of these NCDs do exist, however, these drugs have numerous side effects. Furthermore, despite the prevalence and burden of these disorders, a large proportion of people with such problems do not receive treatment[2]. Treatment remain largely inaccessible predominantly in the developing countries due to their exuberant price tag as well as weaknesses in the health care systems, hence, they depend, even if nominally, on alternative therapies such as traditional herbal medicines.

In certain parts of Africa, traditional medicine remains the most employed method of healthcare

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because of their accessibility to the community[3]. The importance of phytomedicines has been recently sparked scientific investigations, as the therapeutic functionality of medicinal plants is limited. Common plants that are effective and tested against hyperglycaemia are *Panax ginseng (Ginseng), Momordica charantia* (Bitter melon), *Coptis chinensis, Trigonella foenum-graecum* (Fenugreek), *Lagerstroemia speciosa, Gymnemasylvestre, Cinnamomum cassia* (Cinnamon) and *Agaricus campestris* mushrooms[4]. Kamtekar *et al.*,[5] found that plant extracts that are rich in phytochemical secondary metabolites such as flavonoids and phenolics have the potential to control diabetes due to the alpha amylase inhibition potential of these compounds. Odhav *et al.*,[6] found that traditional African vegetables such as *Centella asiatica, Ceratotheca triloba, Cleome monophylla* and others were effective in inhibition of α-amylase. Plant derived compounds such as terpenoids and polyphenolic are known to possess in vitro ACE inhibitory activities[7]. Ranilla *et al.*,[8] found that peppers and spices (*Cuminum cyminum, Zingiber officinale, Curcuma longa and Cinnamomum zeylanicum*) had significant ACE inhibition due to the phenolic compounds present and can significantly aid in lowering hypertension. About 60% of anticancer agents that are currently used come from natural sources[9]. These include vinca alkaloids, taxanes, podophyllotoxin, campothecin, anthracyclines[10,11].

However, the quest to find the ideal anticancer drug, which kills cancer cells while having minimal effect on normal cells, endures. *Hypoxis colchicifolia* is commonly referred to as broad leaved *Hypoxis,* 'inkomfe', 'igudu', 'ingcobo' and 'ilabatheka', in Zulu. It is one of the four most sought after plant species in traditional medicine. *H. colchicifolia* corms are used against barrenness, heart weakness and bad dreams. Infusions of the corm are drunk in small quantities as a tea to stop nausea, vomiting, anxiety, to calm the heart, improve appetite, induce good sleep and even as a treatment for diabetes[12]. *H. colchicifolia* leaves, has not been scientifically validated previously eventhough it is used extensively in traditional medicine. The leaves may contain therapeutic potential like that of the corms. Therefore, this study aimed at investigating the potential biological activity of *H. colchicifolia* leaves and corms. Phytochemical analysis of *H. colchicifolia* has been done as the initial part of the study, and has been used to establish the mode of action of the extracts biological activity[13].

2. Material and Methods

2.1 Collection of Plant Material

Hypoxis colchicifolia was collected and identified using taxonomic keys by the School of Life Science, University of KwaZulu-Natal. The sampling site was located in Mooiriver, KwaZulu-Natal, South Africa with voucher specimens of the authenticated plant material deposited in the Ward Herbarium at UKZN (Westville campus) (Voucher number: Baijnathsn-01).

2.2 Preparation of Plant Material

Fresh as well as dried corms and leaves of *Hypoxis colchicifolia* were washed, cut and allowed to air dry. Plant material was then coarsely ground in an industrial grinder (RetschGmbh, West Germany), and stored in labeled Schott bottles in cool dark condition for further use.

2.3 Extraction of Plant Material

The fresh corms (150g), dried corms (20g) and leaves (20g) were extracted using different solvents (acetone, methanol, distilled water) at the ratio of 1:4 w/v, for 48 h on a rotary shaker and filtered using Whatman No. 1 filter paper. Filtrates were then evaporated using a Buchi rotary evaporator with resulting extract air dried further, with the aqueous extract evaporated at 40^0C in a drying incubator. All extracts formed a solid, glass like extract that was stored in the dark at room temperature till required.

2.4 Anti-diabetic Screening

2.4.1 Alpha Amylase Inhibition Assay

Alpha amylase inhibition was tested using the method by Ranilla *et al.*,[14] with minor modifications. Sodium Potassium Tartrate solution was made by adding 12 g of $KNa_2C_4H_4.4H_2O$ to 8 mL of 2 M NaOH and heated till dissolved. Twenty milliliters of 96 mM 3,5 Dinitrosalicylic acid (DNS) solution was made in distilled water and was heated till dissolved. The DNS solution was then added to the Sodium Potassium Tartrate solution with the addition of 8 mL distilled water. This was allowed to stir in the dark overnight (±16 h). A 20 mM sodium phosphate buffer was made up with 6 mM NaCl. The extracts were suspended in the sodium phosphate buffer (1mg/mL concentration). Starch solution (1%) was made in sodium phosphate buffer. One milligram of 1% soluble starch solution was added to 1 mL of sample (200, 400, 600, 800, 1000 µg/mL) and was incubated for 5 min.

Thereafter 1 mL of 1 unit/mL α-amylase solution made in sodium phosphate buffer was added and incubated for 3 min. DNS solution (1 mL) was added to the reaction mixture and the reaction was thereafter boiled for 15 min at 100^0C. The samples were then cooled to room temperature and 9 mL of distilled water was added. The samples were then transferred to a 96-well plate and read at 540 nm. Absorbance values were converted into percentage Inhibition using the following equation:

$$Inhibition(\%) = \frac{Absorbance\ 540\ (control) - Absorbance\ 540\ (sample)}{Absorbance\ 540\ (control)} \text{ x } 100$$

2.4.2 Alpha Glucosidase Inhibition Assay

The α-glucosidase assay was conducted using the method by Ranilla et al., (2009) with minor modifications. A 50 µL sample (200, 400, 600, 800, 1000 µg/mL) was added to 50 µL of 0.1 M potassium phosphate buffer (pH 6.9) and 100 µL of 1 U/mL α-glucosidase enzyme solution (in 0.1 M potassium phosphate buffer, pH 6.9) was added. This was then incubated at 25°C for 10 min. Following pre-incubation, 50 µL of 5 mM p-nitrophenyl a-d-glucopyranoside solution (in 0.1 M potassium phosphate buffer) was then added. This was further incubated at 25°C for 5 min. The control used was the buffer in place of sample and the blank was the buffer in place of the enzyme. The absorbance was read at 405 nm before and after incubation using a micro plate reader, with percentage inhibition calculated using the following equation:

$$Inhibition(\%) = \frac{\Delta Absorbance_{405}\ (control) - \Delta Absorbance_{405}\ (sample)}{\Delta Absorbance_{405}\ (control)} \text{ x } 100$$

2.5 Anti-hypertension (ACE Inhibition Assay)

The ACE inhibition assay was conducted according to Li *et al.*,[15] and Chen *et al.*,[16] with minor modifications. Twenty microliters of sample (200, 400, 600, 800, 1000 µg/mL) was suspended in sodium borate buffer, 50 µL of 5 mM HHL (in 0.1M sodium borate buffer) and 0.3 M sodium chloride (pH 8.3). This was then pre-incubated at 37°C for 30 min. Thereafter 10 µL (1 U/mL) ACE solution was added to initiate the reaction. This reaction was incubated at 37°C for 30 min.

One hundred microliters of 1 M HCl was added to stop the reaction and absorbance read at 492 nm. The sample blank was buffer in place of enzyme solution and the sample control buffer in place of sample.

$$Inhibition(\%) = \frac{Absorbance_{492}\ (control) - Absorbance_{492}\ (sample)}{Absorbance_{492}\ (control) - Absorbance_{492}\ (blank)} \text{ x } 100$$

2.6 Cytotoxicity Screening (MTT Assay)

Human embryonic kidney (HEK-293), breast cancer (MCF-7) and human lung cancer (A549) cell lines were obtained from the Department of Human Physiology at the University of KwaZulu-Natal, Westville campus and grown at 37°C in a humidified incubator under 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM). The 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to determine the cytotoxicity of the isolates. The MTT assay was conducted according to Dwarka *et al.*,[17] with minor modifications. Briefly, cells (50 µL) (1x10¬2 cells/mL) as well as 50 µL DMEM were seeded in 96-well flat bottom plates and incubated for 24 h at 37°C in a humidified incubator under 5% CO2¬. Cells were then treated with 50 µL of sample extract prepared in 5% DMSO (7.8-1000 µg/mL) and incubated for 24 h. Camptothecin was used as the positive control. MTT reagent (20 µL, 5 mg/mL) was added to the cells and incubated (4 h at 37°C). Finally, 100 µL of DMSO was added to each well in order to solubilize the formazan salt formed. The absorbance was read at 570 nm on a micro plate spectrophotometer (Multiscan Go, Thermo Scientific) and the percentage viability determined using the formula:

$$\%\ Cell\ viability = \frac{Absorbance\ of\ treated\ cells}{Absorbance\ of\ untreated\ cells} \text{ x } 100$$

2.7 Statistical Analysis

Results were analyzed by ANOVA (Graph Pad Prism software, San Diego, CA, USA). All analyses were done in triplicate, mean±standard deviation was calculated. IC_{50} was also calculated using Graph Pad Prism. The lower the IC_{50} concentration, the more potent the extract as a therapeutic.

3. Results and Discussion

3.1 Alpha Amylase Inhibition

The IC_{50} for α-amylase inhibition in descending order are as follows: dried leaf aqueous (392.4 µg/ml), dried corm acetone (391.8 µg/ml), dried corm aqueous (386 µg/ml), dried leaf acetone(366.1 µg/ml), dried leaf methanol (359.3 µg/ml), fresh corm methanol (351.4 µg/ml), fresh corm aqueous (350.9 µg/ml), dried corm methanol (346.5 µg/ml) and fresh corm acetone (337 µg/ml) (Figure 1). The IC_{50} of the positive control acarbose was 515.6 µg/ml.The acetone extract of fresh corms had prominent-amylase inhibition, with the aqueous extract of the leaves having

least effect. All extracts had a greater inhibitory potential than that of the positive control acarbose. There was no significant difference between the results of FCA and FCM, FCA and DCAQ, FCM and DCAQ, DLA and DLM, DLA and DCA; and DLM and DCM. The rest of extracts tested had a significant difference (p<0.0001) with each other and the positive control. Akinrinde *et al.,*[18] found that the aqueous extracts of *Hypoxis argentea* showed no *in vitro* α-amylase inhibition, with no percentage inhibition of the three concentrations tested. However, a study by Alimi and Ashafa[19], on leaf extracts of *Sutherlandia montana*, IC_{50} values ranged between 0.13 and 5.52 mg/mL for α-amylase inhibition, all extracts tested had an improved inhibition than that of acarbose (IC_{50} 0.24 mg/mL), with aqueous extracts of the plant displaying the best inhibition. A similar study by Nair *et al.,* (2013) on methanol extracts of medicinal plants (*Artocarpus altilis, Artocarpus heterophyllus, Cinnamomum zeylanicum* and *Piper betel*) found that α-amylase inhibition by these plants had IC_{50} values ranging between 7.058 and 130.55 µg/mL, with *A. heterophyllus* having the greatest inhibition potential.

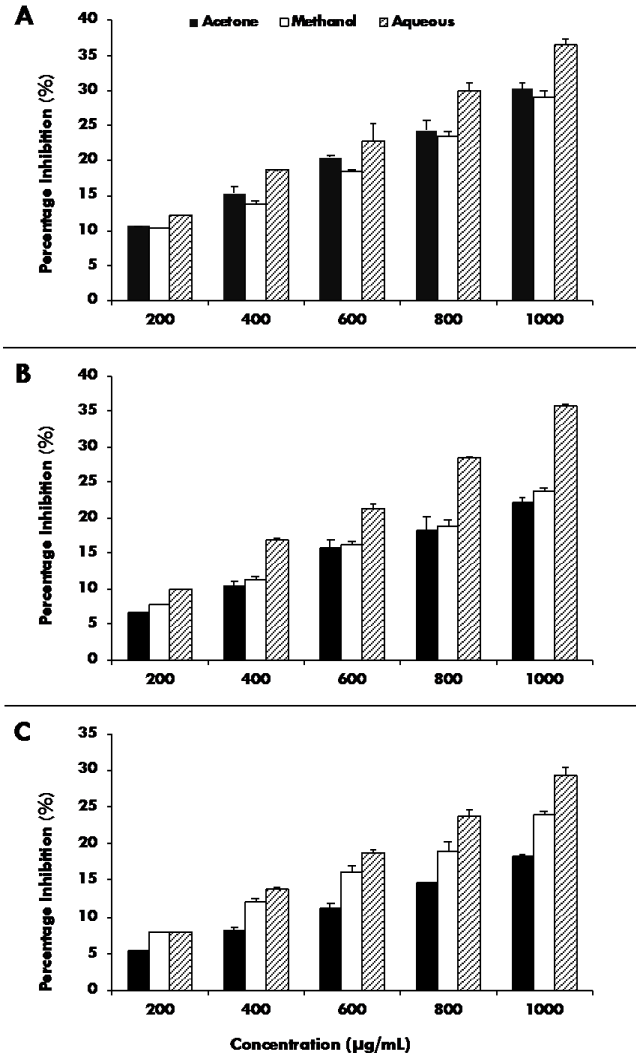


Figure 1. Alpha amylase inhibitory potential of *H. colchicifolia* extracts [**A** - fresh corms; **B** - dried leaves; **C** - dried corms]. Data denotes mean±standard deviation (n=3).

3.2 Alpha Glucosidase Inhibition

The IC_{50} for α-glucosidase inhibition in descending order are as follows: dried leaf aqueous (210.5 µg/ml), dried leaf acetone (152.9 µg/ml), dried corm methanol (130.8 µg/ml), fresh corm methanol (73.83 µg/ml), dried leaf methanol (63.53 µg/ml), fresh corm aqueous (53.89 µg/ml), dried corm acetone (36.67 µg/ml), dried corm aqueous (29.92 µg/ml) and fresh corm acetone (22.06 µg/ml) (Figure 2). The IC_{50} of the positive control acarbose was 118.4 µg/ml. The aqueous extract of dried leaves had the highest α-glucosidase inhibition potential. Fresh corm acetone extracts had the lowest IC_{50}, indicating effective activity. There was no significant difference between FCAQ and DLM, DCA and DCM; and DCA and DCAQ. The rest of extracts tested had a significant difference (p<0.0001) with each other and the positive control. Aqueous extracts of *H. argentea* showed a very low dose dependent α-glucosidase inhibition[18]. In an *in vivo* study by Oguntibeju *et al.,*[20], the methanol extracts of *H. hemerocallidea* corms are effective antioxidant and anti-hyperglycaemic agents, however increased concentrations of the extracts showed negative effects on the kidneys. Alpha glucosidase inhibition by *S. Montana* leaf extracts had IC_{50} values ranging between 0.05 and 0.43 mg/mL, with that of acarbose being 0.31 mg/mL. The decoction extract was most effective. Nair *et al.,*[21] found that medicinal plants tested for α-glucosidase inhibition had good inhibition, with IC_{50} values for the four plants tested ranging between 76.90 and 140.01 µg/mL, similarly a-amylase inhibition in the study, *A. heterophyllus* had the best inhibition potential. Sama *et al.,*[22] found crude ethanol extracts of *Cissus arnottiana* fruit had significant anti-diabetic potential due to the extract inhibition of α-amylase and α-glucosidase at concentrations above 5 mg/mL.

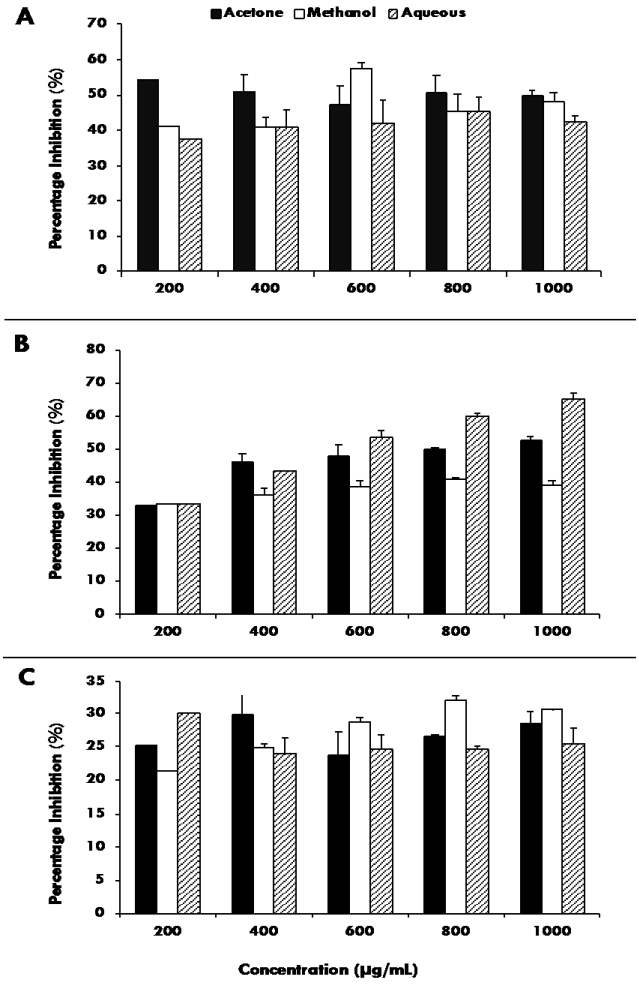


Figure 2. Alpha glucosidase inhibitory potential of *Hypoxis colchicifolia* extracts [**A** - fresh corms; **B** - dried leaves; **C** - dried corms]. Data denotes mean±standard deviation (n=3).

3.3 ACE Inhibition

The IC_{50} for ACE inhibition in descending order are as follows: dried leaf acetone (705.6 µg/ml), fresh corm aqueous (691.2 µg/ml), dried corm acetone (628.2 µg/ml), dried corm aqueous (569.4 µg/ml), dried leaf methanol (542.6 µg/ml), dried leaf aqueous (537 µg/ml), fresh corm acetone (503 µg/ml), fresh corm methanol (439.7 µg/ml) and dried corm methanol (368.2 µg/ml) (Figure 3). The IC_{50} of the positive control captopril was 442.5 µg/ml.The methanol extract of *H. colchicifolia* dried corms has the lowest IC_{50}, denoting optimal dosage for ACE inhibitory potential and the acetone extract of dried leaves had the highest IC_{50} value. Only the methanol extract of fresh and dried corms was more effective than that of the positive control, captopril. There were no significant differences between the results of the following extracts: FCA and FCAQ, FCA and DLM, FCA and DCM, FCM and FCAQ, FCM and DCM, FCAQ and DLM, DLA and DCAQ, DLM and DCM, DCM and DCAQ. The rest of extracts tested had a significant difference (p<0.0001) with each other and the positive control. This mainly denotes no significant difference between fresh corms extracts; no significant difference in between the methanlic extracts of fresh and dried corms. Duncan *et al.*,[23] found that aqueous and ethanolic extracts of leaves and roots of *H. colchicifolia* tested for ACE inhibition produced poor inhibition of between 4-37% inhibitions, with both leaf extracts having a greater inhibition than that of root extracts. Arhin *et al.*,[24] showed a greater ACE inhibitory potential of the methanolic extracts of the leaves of *Tulbaghia acutiloba* with inhibition activity of 76.66 ± 1.65 (IC_{50} 154.23 µg/mL).

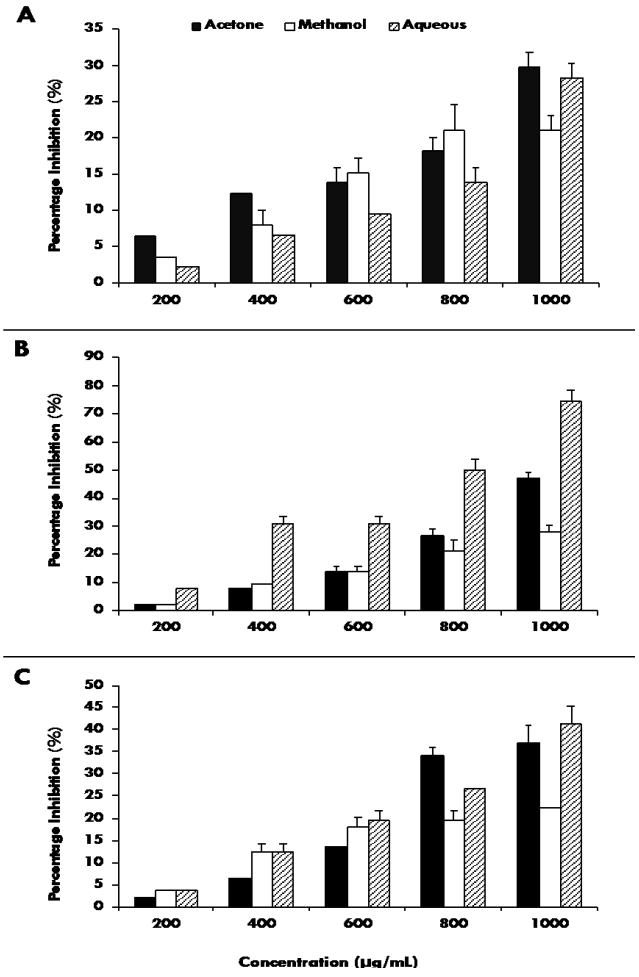


Figure 3. ACE Inhibitory potential of *Hypoxis colchicifolia* extracts [**A** - fresh corms; **B** - dried leaves; **C** - dried corms]. Data denotes mean±standard deviation (n=3).

3.4 MTT Cytotoxicity

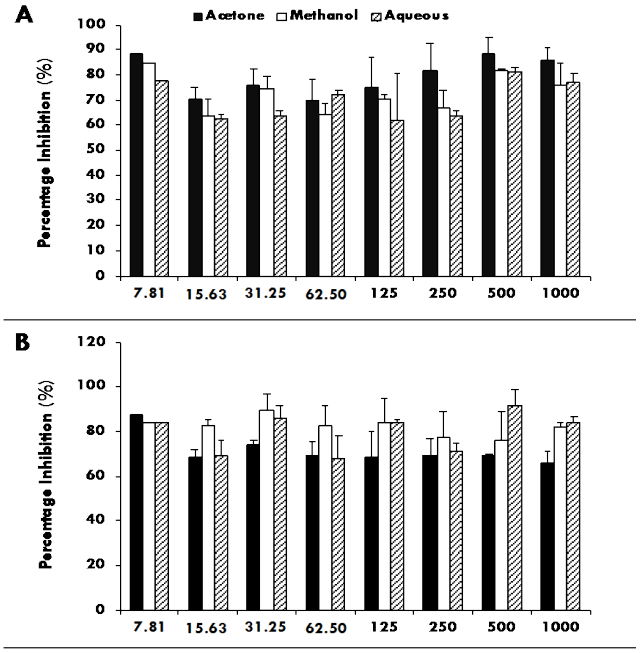
The IC_{50} for HEK-293 inhibition in descending order are as follows: dried leaf methanol (14.16 µg/ml), dried leaf aqueous (11.35 µg/ml), dried leaf acetone (9.02 µg/ml), dried corm aqueous (7.99 µg/ml), fresh corm methanol (7.98 µg/ml), dried corm acetone (7.96 µg/ml), dried corm methanol (7.93 µg/ml), fresh corm aqueous (7.34 µg/ml) and fresh corm acetone (5.39 µg/ml) (Figure 4). The IC_{50} of the positive control camptothecin was 9.06 µg/ml.The acetone extract of *H. colchicifolia* fresh corms produced the greatest cell inhibition and the methanol extract of dried leaves had the lowest cell inhibition in HEK-293 cell line. All extracts tested showed no significant difference when compared to each other and the control.

A study by Madikizela and McGaw[25] showed that the corm extracts of *H. colchicifolia* had an LC_{50} values of 2.48, 0.89 and 0.98 mg/mL against Vero monkey kidney cells for aqueous, ethanol and acetone extracts respectively. A study by Madikizela and McGaw (2018) showed that the corm extracts of *H. colchicifolia* had an LC_{50} values of 2.48, 0.89 and 0.98 mg/mL against Vero monkey kidney cells for aqueous, ethanol and acetone extracts respectively.

The IC_{50} for MCF-7 inhibition in descending order are as follows: fresh corm acetone (9.51 µg/ml), fresh corm aqueous (7.49 µg/ml), dried corm aqueous (7.41 µg/ml), fresh corm methanol (7.28 µg/ml), dried leaf acetone (7.19 µg/ml), dried corm acetone (4.52 µg/ml), dried corm methanol (4.34 µg/ml), dried leaf aqueous (3.83 µg/ml), and dried leaf methanol (3.24 µg/ml) (Figure 5). The IC_{50} of the positive control camptothecin was 8.44 µg/ml.The methanol extract of *H. colchicifolia* dried leaves produced the greatest cell inhibition and the acetone extract of fresh corms had the lowest cell inhibition in MCF-7 cell line. All extracts examined showed no significant difference when compared to each other and the control. In a cytotoxicity screening of African medicinal plants, Steenkamp and Gouws[26] found that aqueous extracts of *H. hemerocallidea* corms stimulated cell growth of DU-145 (prostate carcinoma cells), MCF-12A (non-malignant breast cancer cells) and inhibited the growth of MCF-7 cells. Boukes and van de Venter[27] evaluated the cytotoxicity of *H. hemerocallidea, H. stellipilis* and *H. sobolifera* chloroform corm extracts in HeLa (cervical), HT-29 (colorectal) and MCF-7 (breast) cancer cell lines using the MTT assay. Findings suggest that *H. sobolifera* has the best overall cytotoxic effect against the cancerous cell lines screened, with *H. hemerocallidea* effectively inhibiting HT-29 and *H. stellipilis* having stimulated the growth of HeLa as well as HT-29 cells.

The IC_{50} for A549 inhibition in descending order are as follows: dried leaf aqueous (280 µg/ml), fresh corm aqueous (270.3 µg/ml), fresh corm acetone (228.7 µg/ml), fresh corm methanol (215.9 µg/ml), dried corm methanol (118.9 µg/ml), dried leaf acetone (95.65 µg/ml), dried corm acetone (87.07 µg/ml), dried leaf methanol (68.68 µg/ml) and dried corm aqueous (32.22 µg/ml) (Figure 6). The IC_{50} of the positive control camptothecin was 304.2 µg/ml.

The aqueous extract of *H. colchicifolia* dried corms has the highest cell inhibition and the aqueous extract of dried leaves had minimal inhibition in A549 cell line. These results indicate that the extracts are toxic to cancerous cells while not producing a drastic decrease in normal cells. All extracts tested showed no significant difference when compared to each other and the control. This is in opposition to studiesby Madikizela and McGaw[28] who found that aqueous extracts of corms to be least toxic and had the highest IC_{50} (2480 µg/mL) in non-cancerous Vero African monkey kidney cells. However, when tested in A549, CaCo-2, HELA and MCF 7 in different solvents (acetone, ethanol, hot and cold water), had an IC_{50} ranging from 50-251.95 µg/mL. *Hypoxis colchicifolia* has anticancer potential could be due to glycoside hypoxoside and rooperol activity[26]. In a study by Steenkamp and Gouws[29], corms of *Hypoxis* were found to be non-cytotoxic against prostate cancer cells, breast cancer cells and non-malignant breast cancer cell lines at a concentration of 50 µg/mL.



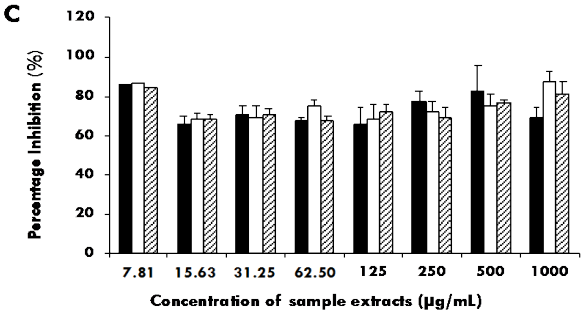


Figure 4. HEK -293 cell line Inhibition by *H. colchicifolia* extracts [**A** - solvent extracts of fresh corms; **B** - solvent extracts of dried leaves; **C** - solvent extracts of dried corms]. Values represent mean±standard deviation of replicate readings (n=3).

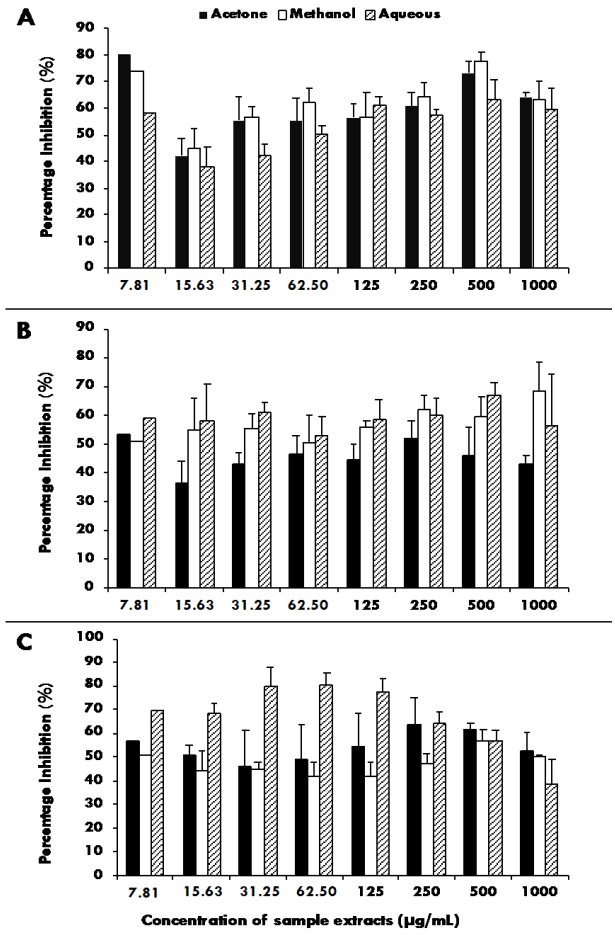


Figure 5. MCF-7 cell line Inhibition by *H. colchicifolia* extracts [**A** - solvent extracts of fresh corms; **B** - solvent extracts of dried leaves; **C** - solvent extracts of dried corms]. Values represent mean±standard deviation of replicate readings (n=3).

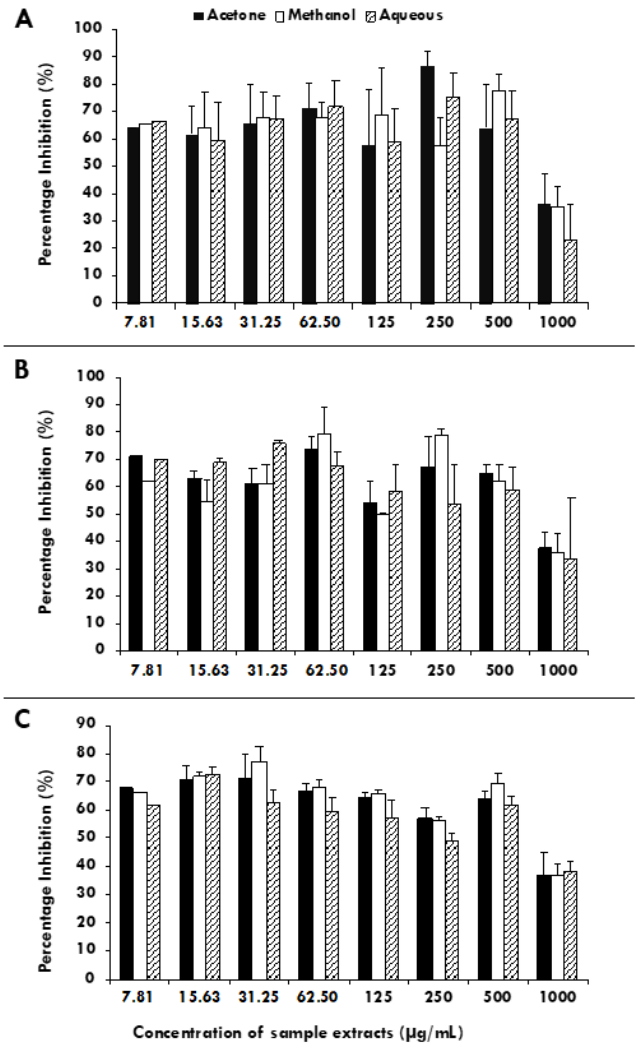


Figure 6. A549 cell line Inhibition by *H. colchicifolia* extracts [**A** - solvent extracts of fresh corms; **B** - solvent extracts of dried leaves; **C** - solvent extracts of dried corms]. Values represent mean±standard deviation of replicate readings (n=3).

4. Conclusion

The fresh corms acetone extract was effective in producing anti-diabetic effects with the dried corm methanolic extract being active in hypertension suppression. Methanol extracts of dried leaves were successful in inhibiting cancerous cell lines while remaining non-toxic to noncancerous cell lines. This study shows that different parts of the plant have different capabilities as a therapeutic and cannot be used interchangeably. Although *H. colchicifolia* has potential to act as a therapeutic, further *in vivo* studies need to be conducted.

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