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# HYPOGLYCAEMIC, PHYTOCHEMICAL AND OTHER PROPERTIES OF ALLIUM CEPA L.

Nazar Sirag[1] B Pharm ; EL-Hadi M.M Ahmed[1] PhD; Hassan EL -Subaki[2] PhD.
1 Faculty of Pharamcy , University of Gezira , Medani.
2. Medicinal and Aromatic Herbs Research Institute , the National Council for Research, Khartoum.

**Abstract**

In the view of the promising potential of hypoglycaemic plants, 19 plants indigenous to Sudan and of common use in Sudanese folk-medicine, were screened for their hypoglycaemic activity. Out of these *Allium cepa L* being the most active, safe and non toxic, available and affordable, hence it had been selected for further investigation.

*Allium cepa* was subjected to chemical tests and chromatographic procedures for the detection of its active components present. Conducted tests and procedures confirmed the presence of sulfur compounds. Comparative chromatographic methods as thin layer chromatography and C–18 high performance liquid chromatography revealed that the aminoacid, cysteine (0.059%) occurs as a major constituent found in Allium cepa.

*Allium cepa* alcoholic extract was found to possess its hypoglycemic effect (36.33 mg/dl – reduction of glycemia) through a synergestic action of its similar sulfur phytoconstituents present, since less activity was exhibited by each of it's fractions (aqueous and organic) obtained when partioned with hexane.

We can conclude that the integrated properties of the active medicinal products especially edible plants with their hypoglycaemic activity, make their use for the control and prevention of diabetes mellitus possible and recommendable. Unstability, similarity in constituents and synergy of their activity give reasons for not to isolate individual compounds from onions and give credits to use them whole as a crude drug or a concentrated extract. Furthermore, descriptive characters, chemical tests and chromatographic profiles obtained in this study could be used as measures to standardize red onion growing in Sudan.

**الملخص:**

تم اختيار البصل لمزيد من الاستقصاء حيث أخضع لتجارب كيميائية وكروماتوغرافية لمعرفة مكوناته الفعاله. أكدت هذه التجارب وجود مركبات الكبريت في البصل. أما طرق المقارنة الكروماتوغرافية مثل كروماتوغرافيا الطبقة الرقيقة وكربون – 18 كروماتوغرافيا السوائل عالية الكفاءة أوضحت أن الحامض الأميني، سيستائين (0,059%) هو المكون الرئيسي الموجود في مستخلصات البصل.

وجد أن مستخلص البصل الكحولي يمتلك أثراً خافضاً للسكر (36,33 ملغم/دل – نقصان في مستوى السكر) من خلال عمل تآزري لمكوناته الكبريتية المتشابهة الموجودة، حيث أن نشاطاً قليلاً أظهرته طبقتاه (المائية والعضوية) عند فصله بمذيب الهكسين.

**يمكننا أن نستنتج بأن تكامل خصائص هذه المنتجات الطبيعية وخاصة الغذائية (edible) منها مع نشاطها الخافض للسكر يجعل من استخدامها لغرض السيطرة والوقاية من داء السكر أمراً ممكناً ومحبذاً. كما أن عدم ثبات، تشابه المكونات والنشاط التآزري تمثل أسباباً لعدم فصل مكونات البصل وتعطي إمتيازاً لأستخدامها في شكل دواء خام أو مستخلص مركز. إن الخصائص الوصفية، التجارب الكيميائية ونتائج الكروماتوغرافيا المأخوذة من هذه الدراسة يمكن أن تستخدم كمقياس لمعايرة البصل الأحمر الذي ينمو في السودان.**

## Introduction:

Diabetes mellitus is a major disease affecting nearly 10% of the population, and inspite of the introduction of hypoglycaemic agents, diabetes and the related complications continue to be a major health problem (Satyanarayana, *et al*., 2004).

Many plant species have been found to be successfully used to manage diabetes including *Allium cepa* which has been thoroughly investigated (Augusti, et al., 1974; Jain and Vyas, 1974; Mathew and Augusti, 1975; Sharma, et al., 1977; Karawya, et al., 1984; Ivorra, et al., 1989; Roman-Ramos, et al., 1995;

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Kumari, et al., 1995; Marles and Farnsworth, 1995; Sheela, et al., 1995; Babu and Srinvasan, 1997;Grover, et al., 2002; EL-Demerdash, et al., 2005).

In this study *Allium cepa* L., being the most active, safe and non toxic, available and affordable among the screened plants, hence it had been selected for further investigation.

**Evaluation and standardization of *Allium cepa* L. (red onion), growing in Sudan.**

The standardization of a herbal drug and a preparation thereof embodies total information and controls that are necessary to guarantee consistency of composition. Therefore standardized drugs of well defined and consistent quality are needed for reliable clinical trials and beneficial therapeutic use.

**a. Part used: Bulb**

**b. Botanical description: Bulb.**

Bulb, is a food storage organ, usually formed underground. The swollen portion consists mostly of fleshy, food-storing scales or thickened modified leaf bases attached to a short flat stem. The leaf base overlap and surround the centre of the bulb. A modified stem forms the base of the bulb, and plant growth occurs from this basal plate. Roots emerge from the underside of the base, and new stems and leaves from the upper side.

Onion, a bulbous plant (*Allium cepa*) cultivated world wide as vegetable, a plant of the family Liliaceae, of the same genous (*Allium*) as the Chive (*Allium schoenoprasum*), garlic *(Allium sativum*), Leak (*Allium porrum*), and shallot (*Allium ascalonium*). These plants are characterized by an edible bulb composed of food-storage leaves that rich in sugar and a pungent oil, the source of its strong taste.

The onion (*Allium cepa*) is a biennial, now grown in many varieties throughout the world as a table vegetable. Common varieties include the strong-flavoured red onion, the milder yellow onion, and the bland white onion. Pearl onions are small white onions used for pickling. Dry onions, which are simply mature onions with a juicy flesh, covered with dry papery skin. Dry onions come in a wide range of sizes, shapes and flavours. They can range from one to four inches in diameter. The above-ground leaves, typically long and tubular, are also eaten.

**c. Culinary uses:**

Although onions have a significant nutritional and medicinal value to the human diet (Health food), they are primarily consumed for their ability to enhance the flavour of other foods (Roderigues, *et al*., 2003)

**d. Phytochemistry:**

The organosulfur compounds responsible for onion's flavour are most likely work through sulfur-sulfur or sulfur-oxygen linkages (Augusti and Sheela, 1996). Oil soluble compounds include sulfides as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide and allyl methyl trisulfide and ajoene. Water-soluble compounds include cysteine derivatives such as 5-allyl cysteine (SAC), S-allyl mercaptocysteine (SAMC), S-methyl cysteine and γ-glutamyl cysteine derivatives. Oil soluble sulfur compounds are odourous, whereas water-soluble compounds are odourless. Oil-soluble compounds are derived from allicine decomposition, resulting in an odorous and highly unstable substance. Allicine is formed by alliin with a reaction catalyzed by the enzyme allinase. The latter is activated by cutting or crushing onion bulbs (Rosa, *et al*., 2004). When Allium are cut or crushed, enzymatic (Allinase) cleavage of S-allyl-L-cysteine sulfoxide (alliin) in the bulbs to produce volatile sulphenic acids, these highly reactive compounds rearrange to form thiosulfinates including thiopropanol-S-oxide which is known as the lachrymatory factor (Ferrary and Auger, 1996), allicine (Larry, *et al*., 1991) and other related compounds (Fig.1).

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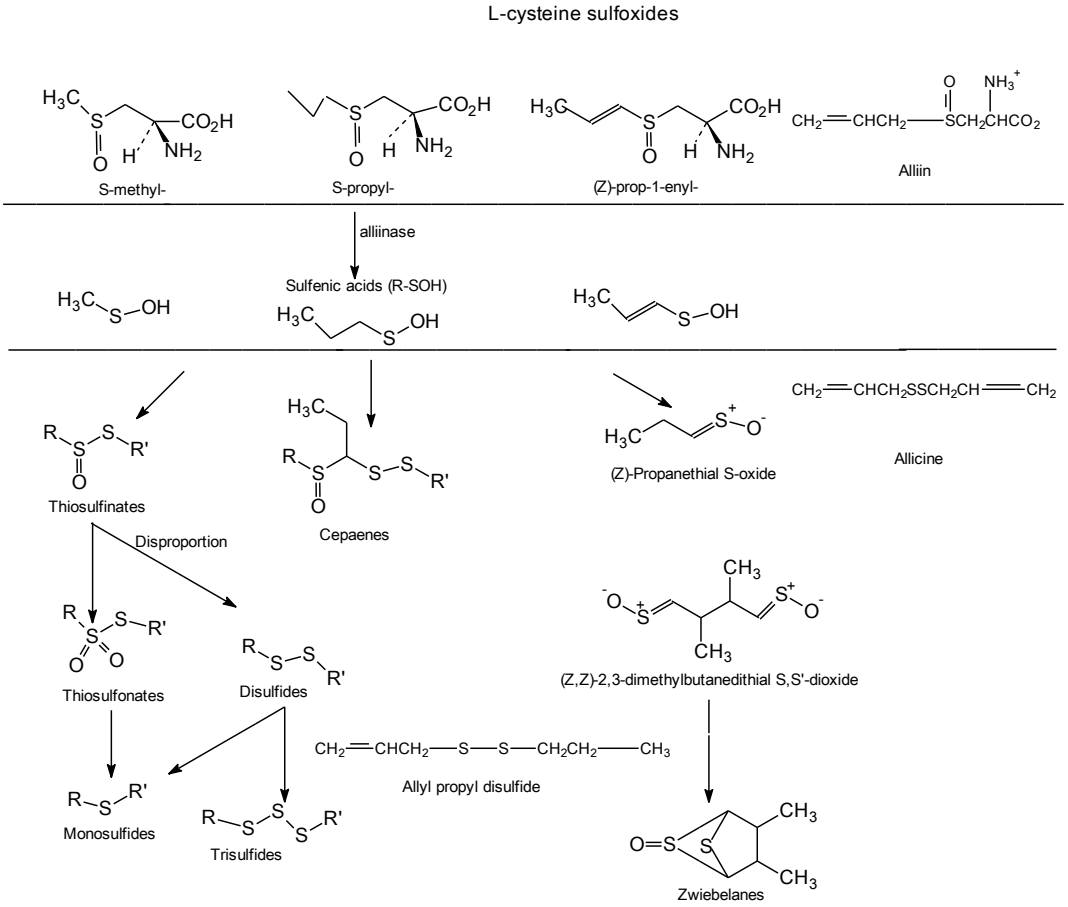


**Fig. 1: The major phytoconstituents in *Allium cepa* L.**

Flavonoids, a group of polyphenolic compounds, widely present in onions, are potent antioxidants (Hertog, *et al*., 1993). Two flavonoids subgroup are found in onion, the anthocyanins, which impart a red/purple colour to some varieties, and flavonols such as quercetin and kaempferol, responsible for the yellow and brown skins of many varieties (Griffiths, *et al*., 2002) .

Onions active constituents, being known, suspected to be unstable, forming a range of similar active phytoconstituents. Analysis of such compounds is therefore difficult because of their unstability and lack the resolution and sensitivity achievable (Larry, *et al*., 1991). However, in this study chemical colour tests for the presence of *Allium* compounds present in different extracts have been conducted as qualitative standards. Moreover, the chromatographic behaviour of these compounds has been documented using thin layer chromatography (TLC) and C18-high performance liquid chromatography methods of separation and determination and were compared to reference samples.

**e. Pharmacology and clinical studies:**

The historical health benefits of dietary consumption of onions have been attributed to organosulfur compounds such as sulfides and thiosulfinates, as well as flavonoids such as quercetin. They have been the focus of much research pertaining to; hypoglycemic, hypolipidemic and antibacterial potentials of onions (Kendler, 1987; Ivorra, *et al*., 1989; Hughes and Lawson, 1991; Lata, *et al*., 1991; Roman Ramos, *et al*., 1995; Sheela, *et al*., 1995; Zohri, *et al*., 1995; EL-Demerdash, *et al*., 2005) and antioxidant activity with beneficial effects on cardiovascular and immune systems, inflammatory conditions and cancer prevention (Kendler, 1987; Wagner, *et al*., 1990; Guo, *et al*., 1994; Chisty, *et al*., 1996; Hatono, *et al*., 1996).

Biological hypoglycaemic activity of *Allium cepa* L. is the most ethnomedical use, practiced by different nations. Many studies have been reported (Jain and Vyas, 1974; Mathew and Augusti, 1975; Sharma , *et*

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*al*., 1977; Ivorra, *et al*., 1989; Roman-Ramos, *et al*., 1995; Sheela, *et al*., 1995; Marles and Farnsworth, 1995; Kumari, *et al*., 1995; Babu and Sirnvasan, 1997; Grover, *et al*., 2002).

We reported here, the hypoglycaemic effects and phytochemical properties of *Allium cepa* L. different extracts.

## MATERIALS AND METHODS

**Plant material**

*Allium cepa* (red onion), newly collected was purchased from local market in Wad Medani.

**Reagents:**

Ninhydrine (Freshly Prepared) as: 0.2 grams dissolved in 25ml chloroform and 25ml butanol. Benedict's solution: B.P (Freshly Prepared) was also used.

**Methods:**

**Extraction of plant material**

Hundred grams of coarsely powdered onions were prepared by maceration using 70% ethanol for 48 hours. Some of the concentrated extract was then fractionated with hexane to obtain hexane and aqueous fractions which were further dried at room temperature. Working solutions (1g/4ml) were made for alcoholic extract and for each fraction using distilled water and kept into a refrigerator for biological and phytochemical tests.

Hundred grams of fresh onions were prepared by blending for 1 minute, allowed to stand for 10 minutes and then filtered for phytochemical investigation.

**Thin layer chromatography (TLC):**

TLC plates were prepared using silica gel G type 60 with $CaSO_4$ as binder. Extracts and standard samples were chromatographed on TLC plates, using butanol, acetic acid and water (BAW) in a ratio 4:1:1 as solvent system, ninhydrine and iodine vapour were used as locating reagents (Table 1).

**C18-High performance liquid chromatography (HPLC):**

HPLC separation of sulfur compounds was carried out on C18- HPLC coloumn ODS 4.6mm X 150mm 5mic (Sykam, S1122 solvent delivery system; S3210 UV/visible detector, Germany). a): Methanol and water (50:50) were used as mobile phase at flow rate of 1ml/min at ambient temperature. UV was operated at 210 nm (Ferrary and Auger, 1996). In this method, 5mg of cysteine, 0.1 ml of sulfides and 10% solution of *Allium cepa* ethanolic extract were dissolved separately in 1ml mobile phase and 20 µl were then injected and/or b): Acetonitrile and water (3:97) of pH adjusted to 3.0 with concentrated phosphoric acid, at flow rate 1.0 ml/min where UV set at 220 nm (BP.,2000). In this method, 10mg cysteine was dissolved in 1ml of 1 M hydrochloric acid. Water was added to 100 ml, and 20 µl from both ethanolic extract and cysteine solutions were then injected.

**Hypoglycemic assay**

**a. Studies on normal fasting animals:**

Rabbits weighing 1 – 1.5 kg were purchased from local market. They were housed in a clean animal house and subjected to intensive nutritional programme. They are divided into groups each of 6 rabbits and submitted to fasting for 16h before each study. The sixteen hours fasting rabbits received water, glibenclamide (5mg/kg) and/or 1g/4ml/ body weight of the ethanolic extracts.

**b. Blood sampling:**

After the gastric administration of water (4ml/kg), glibenclamide (5mg/Kg) and/or ethanolic extract, hexane and aqueous fractions of the ethanolic extract (1g/4ml/Kg), blood samples were collected from the marginal vein of the left ear at 0h, 1h, 2h, and 4h.

**c. Biological assay for normal fasting rabbits:**

After the gastric administration of water , glibenclamide (5mg/Kg) and/or ethanolic extract (1g/4ml/Kg), blood samples were read on Glucomen (A. Menarini, Italy) to determine glucose concentration (Table 2).

**d. Biological assay for glucose tolerance tested rabbits:**

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Glucose tolerance tests had been performed for ethanolic extract (Table 3). For this purpose 50% Dextrose (4ml/Kg) was infused subcutanously. The infusion was done at 0h and 1h. Rabbits received water (4ml/Kg), glibenclamide (5mg/Kg) and/or ethanolic extract, hexane and aqueous fractions of the ethanolic extract (1g/4ml/Kg).

**Statistical analysis:**

Data were analysed using one way ANOVA.

**Results and Discussion**

*Allium* species particularly bulbs and cloves parts are known to be used for their culinary or medicinal values, for being rich in sulfur containing phytoconstituents which are the most characteristic and responsible for the smell of *Alliums*. Some of these sulfur compounds as sulphides, thiosulphinates, sulfenic acids and cysteine sulfoxides, had been isolated and identified (Larry, *et al*., 1991).

Generally, separation of thiosulfinates in Alliums homogenates for being unstable, lacks the resolution, sensitivity and quantitation achievable especially by modern analytical HPLC methods (Larry, *et al*., 1991) or by infrared analysis (Shriner, *et al*., 1980).

In this study, chemical tests for the presence of RSH sulfides (thiols/mercaptans), disulfides and cysteine was confirmed when their characteristic identifying colour tests were carried out using Benedict's and/or mercuric chloride ($HgCl_2$) reagents. Both alcoholic and water extracts gave yellowish brown precipitate with Benedicts which was changed on boiling to oily heavy yellow, while hexane fraction gave no precipitate with Benedicts reagent, but when treated with $HgCl_2$ a white precipitate was formed on boiling. The alcoholic extract and aqueous fraction gave oily yellow precipitate with $HgCl_2$ which changed on boiling to faint yellow.

The results obtained were found to be similar to those described by Shriner, *et al*., (1980) and confirming the presence of sulfur compounds in *Allium cepa* extracts, among which, cysteine, a sulfur aminoacid, was the dominant phytoconstituent.

| Compound | Rf value | Colour of the band (λ 254) |
|---|---|---|
| 1. | 0.095 | - |
| 2. | 0.19 | - |
| 3. | 0.26 | - |
| 4. | 0.36 | - |
| 5. | 0.48 | Dark brown |
| 6. | 0.58 | - |
| 7. | 0.81 | Brown |
| 8. | 0.98 | Yellow |

**Table 1: TLC Details of *Allium cepa* L.**

Detailed comparative TLC profile showed that cysteine (4) and compounds (1–3) were disclosed as pink spots using ninhydrine as positive test for amines, while sulfides and disulfides (5 -8) acquired dark brown spots at the solvent front when exposed to iodine vapour and yellow fluorescence at 254nm (Table 1).

It was also found that freshly prepared extract obtained whether by water or 70% alcohol was made of the same compounds (2, 4, 5, 7 and 8), while extract obtained by maceration was made of compounds (1 – 8), in which the occurrence of more compounds may be attributed to hydrolysis which has been shown in the hydolysed cysteine TLC profile. Furthermore, it was also noticed that hexane fraction of the alcoholic extract contained the sulfides, while cysteine and similar components of its degradative products were found to exist in the aqueous fraction only.

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In a reversed – phase C18-HPLC comparative separation of 10% ethanolic extract of *Allium cepa* was performed at UV 210nm and methanol/water (50:50) mobile phase using pure standards of cysteine, allyl sulfide, allyl disulfide and propyl disulfide. Only cysteine (with retention time, 1.95) was detected as small peak, while others were not separable. In detecting cysteine, the area was not calculated or ignored being of negligible value. On the adoption of the method described by the BP (2000) for acetylcysteine , in which a mobile phase of acetonitrile/water (3:97) was used, a remarkable peak arised (retention time, 2.40) in both tested extract and standard solution with a recognized area. The percentage occurrence of cysteine in our sample was calculated quantitatively to be 0.059%.

The hypoglycaemic effect in normal animals was conducted as decribed by Tayyaba Zia, *et al*., (2001). Results of which, in Table 2 show the effect of *Allium cepa* ethanolic extract on blood glucose level after gastric administration at a dose of 1g/kg body weight in normal fasting rabbits. Values are the mean blood glucose levels ± SEM and P-value changes as compared to water control.

High hypoglycaemic activity was caused by *Allium cepa* ethanolic extract which showed a decrease in blood glucose level as 36.33mg/dL with P-value <0.05 (Table 2).

**Table 2: Effect on blood glucose levels in normal rabbits after gastric administration of glibenclamide and *Allium cepa* ethanolic extract.**

| Preparations | Glucose level (mg/dl) | | | | Reduction of Glycaemia (mg/dl) | P. value |
|---|---|---|---|---|---|---|
| | 0h | 1h | 2h | 4h | | |
| Water | 90.67±6.07 | 181.67±10.15 | 342.33±22.36 | 92.67±10.49 | - | 0.000 |
| Glibenclamide | 85.0±5.46 | 80.0±4.08 | 50±2.04 | 53.33±4.17 | 31.67 | 0.000 |
| Allium cepa | 98.66±9.57 | 101±15.15 | 66±16.62 | 62.33±10.90 | 36.33 | 0.011 |

The ethanolic extract, hexane and aqueous fractions of *Allium cepa* were tested for hypoglycaemic effect using glucose tolerance test (G.T.T) with gastric administration of water, glibenclamide and/or ethanolic, hexane and aqueous fractions of ethanolic extract (Table 3). The hexane and aqueous fraction obtained from the ethanolic extract produced mild hypoglycaemic effect in reducing the hyperglycemic peak compared to the hypoglycaemic activity produced by the mother extract, (Fig. 2). The variation in hypoglycaemic activity of the two fractions of *Allium cepa* ethanolic extract coincides with and validates what had been reported by Evans (2002), that onions active constituents, being known, suspected to be unstable, forming a range of similar active phytoconstituents and with synergestic and polyvalent action.

The hypoglycaemic effect produced by *Allium cepa* (red onion) was not different from results reported previousely (Roman-Ramos, *et al*., 1995; Grover, *et al*., 2002; EL-Demerdash, *et al*., 2005).

**Table 3: Glucose tolerance test (GTT) in healthy rabbits after gastric administration of water, glibenclamide, *Allium cepa*, (ethanolic, hexane and aqueous fractions).**

| Study/preparation | Blood glucose mg/dl% | | | |
|---|---|---|---|---|
| | In fasting | 60 min | 120 min | 240 min |
| Water | 90.67±6.07 | 181.67±10.15 | 342.33±22.36 | 92.67±10.49 |
| Glibenclamide | 117.67±18.78 | 123±5.69 | 151.33±19.57 | 61±5.51 |
| Allium cepa (ethanolic F.) | 108±8.75 | 157±1.16 | 188.67±16.20 | 96.67±4.91 |
| Allium cepa (hexane F.) | 102.67±4.67 | 216.67±20.92 | 291.67±33.42 | 152.33±17.54 |
| Allium cepa (aqueous F.) | 91.33±4.98 | 311±23.21 | 331±42.39 | 186.33±27.77 |

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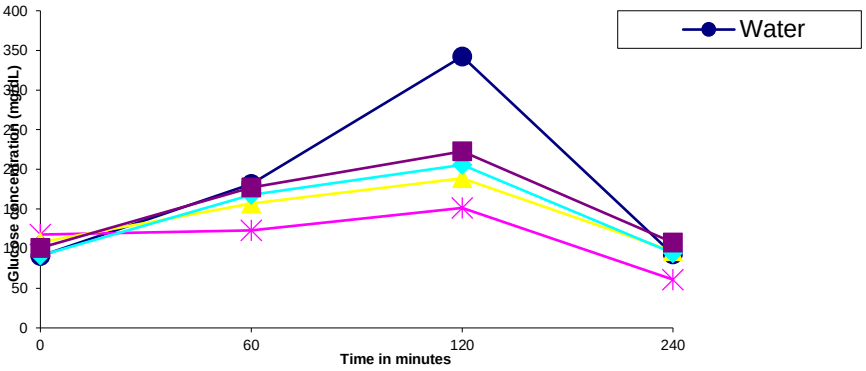


**Fig. 2: Glucose tolerance curves in healthy rabbits with gastric administration of water, glibenclamide, ethanolic, hexane and aqueous fractions of *Allium cepa*.**

## CONCLUSION:

1) The integrated properties of the active medicinal products especially edible plants (vegetables) with their hypoglycaemic activity make their use for the control and prevention of diabetes mellitus possible and recommendable.

2) Unstability, similarity in constituents, and synergy of their activity, give reasons for not to isolate individual compounds from onions and many other herbs and/or extracts and give credits to use them whole as crude drugs or as prepared extracts.

3) Data obtained from the macro-and microscopical characteristics, chemical colour tests, chromatographic profiles and biological assays could be used to assess and standardize *Allium cepa* (red onion) growing in Sudan.

**Recommendations:**

1. *Allium cepa* when medicinally used for its hypoglycaemic activity and due to unstability and similarity in phytoconstituents, it is recommended to use it as a whole crude vegetable and/or prepared aqueous extract.
2. Phytochemical and biological data obtained from this study could be implemented to assess *Allium cepa* (red onion) growing in Sudan.

**Corresponding author:**
EL-Hadi M.M. Ahmed , Faculty of Pharmacy , University of Gezira , Sudan.

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