Phytochemical analysis and evaluation of antioxidant potential of ethanol extract of Allium cepa and ultra-high homoeopathic dilutions available in the market: A comparative study

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Abstract

Introduction: As per statistics available with the WHO, 80% of the earth’s population rely on traditional medicine for their primary health-care needs and most of this therapy involves the use of plant extracts and their active components. Objectives: To confirm the presence of alkaloids, fats, steroids, tannins, flavonoids, sugars, amino acids and saponins in Allium cepa extract. Method: A qualitative phytochemical analysis has been performed to confirm the presence of these components in Allium cepa extract and its homoeopathic formulations (mother tincture [MT], Allium cepa 30C and Allium cepa 200C). The total phenolic content, flavonoid content and antioxidant potential of the prepared extract and the various homoeopathic formulations have also been studied. Results: Presence of various phytochemicals such as flavonoids, alkaloids, saponins, steroids, carbohydrates and amino acids have been confirmed in the prepared extract as well as the MT. According to calculations using Avogadro’s limit, preparations above 12C dilution should have no source material present. It is interesting to note that even though the formulations Allium cepa 30C and 200C are considered ultra-high dilutions, they gave a positive result for many phytochemicals. The total phenolic content, flavonoid content and antioxidant potential of the prepared extract and various homoeopathic formulations have also been studied. Conclusion: The positive qualitative and quantitative results also reinforce the growing belief that Homoeopathy is not just a placebo effect but a ‘Smart medicine’ which may be working on the nanoscale. More research is required to understand various aspects.

Keywords: 2,2-diphenyl-1-picrylhydrazyl, Allium cepa extract, Homoeopathy, Mother tincture, Nanomedicine, Phytochemical analysis

Introduction

Traditional medicine is the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, treatment or improvement of physical and mental illness.[1] The WHO has developed and launched the traditional medicine strategy 2014–2023 in response to the World Health Assembly resolution on traditional medicine (62.13). The strategy aims to support member states in developing proactive policies and implementing action plans that will strengthen the role traditional medicine plays in keeping populations healthy. Allium cepa (Onion) is one of the most important commercial condiment vegetables grown and consumed not only in India but also worldwide. It is known that onions may help guard against many chronic diseases and are highly valued for their therapeutic properties. It has been reported to be very high in quercetin content. It is said to be particularly useful in the treatment of patients suffering from running eyes and nose. The onions ability to relieve congestions, especially in the lungs and bronchial tract is well documented.[2] Onions contain large amounts of flavonoids such as quercetin which is abundant in onions and protects against cataracts, cardiovascular diseases and cancer.[3,4] Organosulfur compounds present in it have been linked to lowering blood pressure and cholesterol levels.[5] It is known to possess anthelmintic, anti-inflammatory, antiseptic,
antispasmodic, carminative, diuretic, expectorant, febrifuge, hypoglycaemic and hypotensive properties.\[6\]-\[7\] Onion relieves stomach upset and other gastrointestinal disorders and also strengthens the appetite.

Homoeopathy is a time-tested two-century-old empirical system of healing. Homoeopathic medicines are prepared through a characteristic process known as potentization, where serial dilutions are performed with strong strokes at each step of dilution.\[8\] Homoeopathic pharmacists prepare medicines from various biologically active substances that belong to two main groups: organic materials (plants, animal products and nosodes) and inorganic substances (synthetic chemicals, metal alloys and natural and synthetic ceramic materials).\[9\] These bioactive materials are subjected to specific manufacturing procedures whereby they are dissolved in water and alcohol. To become homoeopathic remedies, medicinal compounds are diluted hundreds, even thousands of times to reduce their toxicity and to ensure that they are biologically active and compatible with the processes of human physiology. The major objection to homoeopathic medicine is that the doses of medicine prescribed in some cases are too dilute (beyond Avogadro limit) for any active ingredient to be present. The medicines, therefore, receive criticism on their activity and are termed as having a placebo effect. A further examination of dilution to establish that homoeopathic medicines may not be as dilute as a simplistic application of Avogadro’s Principle but retains the original source substance due to surface effects has been explained by some mathematical models based on Langmuir equation.\[10\]

New evidence is emerging on the nature and properties of high dilution of homoeopathic medicines. These findings indicate that the science of Homoeopathy is a form of Nanomedicine, with the medicines capable of initiating changes in the physiological and biochemical dynamics of the animal as a complex adaptive system.\[11\] Several lines of evidence suggest that homoeopathic high dilutions do have pharmacological action.\[11\]

In the present work, we have prepared Allium cepa extract, studied the antioxidant activity, measured the total flavonoid and phenolic content, carried out its phytochemical analysis and compared it with the mother tincture (MT) of Allium cepa and homoeopathic dilutions 30C Allium cepa and 200C Allium cepa.

**Methodology**

Allium cepa MT, 30C and 200C (Dr. Reckeweg) have been used for all studies. All other chemicals have been procured from Sigma-Aldrich. All reagents have been prepared following standard protocols. Allium cepa MT has been used as the positive control.

**Preparation of onion extract**

The onion bulb was washed with freshly prepared sterile distilled water. The outer covering of the bulb was manually peeled off, and the fleshy part of the onion was rewash with freshly prepared sterile distilled water and weighed. The onion bulb was cut into small parts and squashed. To the squashed preparation, 90% ethanol (200 mL) was added for 8 h with 10 min interval shaking. The extraction was filtered using muslin cloth and Whatman No. 1 filter paper. The filtrate was evaporated at 45°C to dryness and was kept in a sterile bottle and refrigerated until use.

**Determination of the total phenolic content**

The concentration of phenolics in the samples was determined using spectrophotometric method of Singleton and Rossi.\[12\] The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. A 1 mg/mL methanol solution of the extract was prepared for analysis. The reaction mixture was prepared by mixing 0.5 mL of methanol solution of extract/samples, 2.5 mL of 10% Folin-Ciocalteu’s reagent in water and 2.5 mL 7.5% NaHCO₃. The blank is prepared by mixing 0.5 mL methanol, 2.5 mL 10% Folin-Ciocalteu’s reagent dissolved in water and 2.5 mL of 7.5% of NaHCO₃. The samples were incubated in a thermostat at 45°C for 45 min and the absorbance determined spectrophotometrically at λmax 765 nm. The same procedure is repeated for the standard solution of gallic acid and the calibration line is constructed.

Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration line, and the content of phenolics in the samples was expressed in terms of gallic acid equivalent (mg of GA/mL of sample).

**Determination of the total flavonoid content**

The total flavonoid content was measured with the aluminium chloride colorimetric assay.\[13\] One millilitre of methanol and 1 mL of standard quercetin solution (5, 10, 20, 40, 60, 80 and 100 µg/mL) were added to the test tubes. This was followed by 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution in each. A volume of 0.3 mL of 10% aluminium chloride was added after 5 min followed by 2 mL of 1 M sodium hydroxide. The volume was made up to 10 mL with distilled water and the contents were mixed well. An orange yellowish colour developed. The absorbance was measured at 510 nm spectrophotometrically. The samples were prepared in triplicate. A calibration curve was plotted using the values for standard quercetin. The data of total flavonoid content were expressed as milligram of quercetin equivalents/mL of sample.

**Determination of the antioxidant potential**

The ability of the plant extract/sample to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals is assessed by the standard method.\[14\] A simple method that has been developed to determine the antioxidant activity of foods utilises the stable DPPH radical. The odd electron in the DPPH free radical gives a strong absorption maximum at 517 nm and is purple in colour. The colour turns from purple to yellow as the molar absorbptivity of the DPPH radical at 517 nm reduces when the odd electron of DPPH radical becomes paired with hydrogen from a free radical scavenging antioxidant to form the reduced DPPH-H. The resulting decolourisation is
stoichiometric with respect to number of electrons captured. The samples are prepared in triplicate for each analysis and the mean value of absorbance is obtained. The stock solution of the extract was prepared in methanol to achieve the concentration of 1 mg/mL. Dilutions were made to obtain concentrations of 1000, 900, 800, 700, 600, 500 and 400 µg/mL. Diluted solutions (1 mL each) were mixed with 1 mL of methanolic solution of DPPH in concentration of 1 mg/mL. After 30 min incubation in darkness at room temperature (23°C), the absorbance was recorded at 517 nm. Control sample contained all the reagents except the extract. Percentage inhibition was calculated and IC₅₀ values were estimated from the percentage inhibition versus concentration plot.

Qualitative phytochemical analysis
Plants contain many bioactive chemical substances that produce definite physiological and biochemical actions in the human body. These bioactive constituents are alkaloids, tannin, flavonoids, phenolic compounds, etc. [15,16] Since Allium cepa has received considerable attention in recent years due to diverse pharmacological properties including antioxidant and antitumor activity, a preliminary phytochemical study to determine the phytoconstituents present was undertaken for the extract prepared and the homoeopathic formulations. The ethanol extract was considered suitable since the homoeopathic formulations are in ethanol medium. All tests were performed on the blank. The MT is used as the positive control.

Test for steroids
Salkowski reaction
To the different sample solutions, chloroform was added followed by concentrated sulphuric acid along the sides of the tube. A red-brown colouration indicated the presence of steroids.

Test for alkaloids
The samples were dissolved separately in 1% HCl. One millilitre of the solution was taken and a few drops of the following reagents were added and various tests performed.

Dragendroff’s test
One millilitre of Dragendroff’s reagent was added to the different sample solutions. Formation of reddish brown precipitate indicated the presence of alkaloids.

Mayer’s test
One millilitre of Mayer’s reagent was added to the different sample solutions. Formation of cream colour precipitate indicated the presence of alkaloids.

Hager’s test
One millilitre of Hager’s reagent was added to the different sample solutions. Formation of yellow colour precipitate indicated the presence of alkaloids.

Wagner’s test
One millilitre of Wagner’s reagent was added to the different sample solutions. Formation of reddish brown precipitate indicated the presence of alkaloids.

Test for tannins
Ferric chloride test
Different sample solutions were treated with ferric chloride solution; appearance of blue and green colours indicated the presence of hydrolysable and condensed tannins.

Lead acetate test
Small quantity of the sample solutions was dissolved in distilled water and 10% lead acetate solution was added to them, a white precipitate indicated the presence of phenolics and tannins.

Test for flavonoids
Alkaline reagent test
To the different sample solutions, a few drops of sodium hydroxide solution were added. Formation of intense yellow colour, which turned colourless after addition of few drops of dilute hydrochloric acid, indicated the presence of flavonoids.

Shinoda test
To the different sample solutions, a few magnesium turnings were added followed by a few drop of concentrated hydrochloric acid. Appearance of a crimson red colour indicated the presence of flavonoids.

Test for carbohydrates
Molisch’s test
The solution to be tested are mixed with a small amount of Molisch’s reagent (alpha-naphthol dissolved in ethanol) in a test tube and mixed well. A small amount of concentrated sulphuric acid was slowly added down the sides of the sloping test tube. Appearance of purple ring at the junction indicated the presence of carbohydrates.

Fehling’s test (for reducing sugars)
The sample solutions are mixed with a small amount of Fehling’s solution and heated. Appearance of a red precipitate indicated the presence of a reducing sugar.

Test for saponin glycosides
Froth formation test
A small quantity of the samples was diluted with 20 mL of distilled water and shaken vigorously; formation of 1 cm layer of foam which is stable for 10 min indicated the presence of saponins.

Test for amino acids
Ninhydrin test
A solution of ninhydrin in ethanol is added to the sample solutions. Appearance of a purple colour indicated the presence of amino acids.

Test for cardiac glycosides
Keller–Killiani test
Glacial acetic acid (0.4 mL) and a few drops of 5% ferric chloride solution are added to the sample solutions. Concentrated sulphuric acid (0.5 mL) is added along the side of the test tube carefully. The formation of blue colour
in the acetic acid layer confirmed the presence of cardiac glycosides.

**Test for anthraquinone glycosides**

*Hydroxyanthraquinone test*

To 1 mL of the samples, a few drops of 10% potassium hydroxide solution were added. The formation of a red colour confirmed the presence of anthraquinone glycosides.

**Test for proteins**

*Biuret test*

To 2 mL of the sample solutions, 5 drops of 1% copper sulphate solution are added followed by 2 mL of 10% NaOH. The contents are mixed thoroughly. Formation of a purple or violet colour confirmed the presence of proteins.

### Results

**Total phenolic content**

The total phenolic contents of the samples using Folin–Ciocalteu’s reagent are expressed in terms of gallic acid equivalent in Figure 1. The reagent which is a mixture of phosphotungstic acid and phosphomolybdic acid which after oxidation of the phenols is reduced to a mixture of blue oxides of tungsten and molybdenum. The blue colouration produced has a maximum absorption in the region of 765 nm and is proportional to the total quantity of phenolic compounds originally present. The total phenolic content for *Allium cepa* extract and various homeopathic formulations are presented in Table 1 and Figure 2.

**Total flavonoid content**

The concentration of flavonoids in *Allium cepa* extract was determined using spectrophotometric method with aluminium chloride. The standard curve for quercetin is provided in Figure 3. The total flavonoid content was expressed in terms of microgram quercetin equivalent in Figure 4. The results for the total flavonoid content for *Allium cepa* extract and various homeopathic formulations are presented in Table 2.

**Antioxidant potential**

The scavenging activity percentage (AA%) was determined using equation 1 according to Mensor *et al.*[^14^] Antioxidant (DPPH scavenging) activity of *Allium cepa* extract is presented as percentage of DPPH radicals inhibition in Figure 5. Ascorbic acid is used as a reference. The IC<sub>50</sub> value of the extract is found to be 915 µg/mL. The DPPH radical scavenging activity for *Allium cepa* extract and various homeopathic formulations is presented in Table 3. *Allium cepa* extract and MT show appreciable antioxidant activity while the homeopathic formulations 30C and 200C do not show any appreciable antioxidant activity.

\[
\text{AA} \% = 100 - \left( \frac{[A_{\text{sample}} - A_{\text{blank}}]}{A_{\text{control}}} \times 100 \right) / A_{\text{control}}
\]  

(1)

**Phytochemical analysis**

Although quantitative analysis is more sensitive to very small amount of the active constituents, qualitative analysis is the first step in any analysis. The qualitative analysis of onion extract and *Allium cepa* preparations available in the market showed the presence or absence of phytochemicals as shown in Table 4. The phytochemical evaluation of the various phytoconstituents is shown in Figure 6. We see the presence of various important bioactive constituents in the sample solutions of ultra-dilute homeopathic formulations. This is a very important finding given that mathematically...
we do not expect any active material present in these ultra-dilute solutions. More research needs to be carried out in this direction.

**DISCUSSION**

Our qualitative and quantitative studies show that homeopathic preparations of *Allium cepa* MT, 30C and 200C show the presence of many important phytochemicals even in the ultra-dilute preparations. This study validates the claim made by a scientist that homeopathic formulations retain the starting material in spite of being ultra-dilute. This has been attributed to the succussion process followed to prepare different potencies. The succussion given to the liquid mass is expected to produce particles of varied shapes and sizes due to shearing forces generated during the pounding of the liquid container against an elastic stop. Mathematical modelling and study of physiochemical properties suggest that surface effects are responsible for the transfer of active ingredients during successive dilutions such that even ultra-dilute solutions show retention of active ingredients.

**CONCLUSION**

The controversy regarding Homoeopathy medicines having high potencies i.e., 30C and 200C which involve huge dilution...
Arora, et al.: Qualitative and quantitative evaluation of ultra-high homoeopathic dilutions


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Conflicts of interest
None declared.

References
Arora, et al.: Qualitative and quantitative evaluation of ultra-high homoeopathic dilutions

Phytochemical Analyse and Bewertung des Antioxidanspotentials von Ethanol-Extrakt von Allium cepa und ultra-hohen homöopathischen Verdünnungen auf dem Markt verfügbar: Eine vergleichende Studie

Einführung: Nach den Statistiken, die mit der WHO zur Verfügung stehen, verlassen sich 80% der Erdbevölkerung auf die traditionelle Medizin für ihre primären Gesundheitsversorgung und die meisten dieser Therapie beinhaltet die Verwendung von Pflanzenextrakten und deren aktiven Komponenten.


Fazit: Die positiven qualitativen und quantitativen Ergebnisse verstärken auch den wachsenden Glauben, dass Homöopathie nicht nur ein Placebo-Effekt ist, sondern eine "intelligente/schlau Medizin", die auf der Nanoskala arbeiten kann.

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Análisis fitoquímico y evaluación del potencial antioxidante del extracto de etanol de Allium cepa y las ultra diluciones homeopáticas en el mercado: estudio comparativo

Resumen

Fundamento: Conforme a la estadística disponible por la OMS, el 80 % de la población de la tierra confía en la medicina tradicional para sus necesidades de atención primaria y la mayoría de estas terapias implica el uso de extractos de plantas y sus componentes activos.

Objetivos: Se efectuó un análisis fitoquímico cualitativo para confirmar la presencia de diferentes componentes como alcaloïdes, grasas, esteroides, taninos, flavonoides, azúcares, aminoácidos y saponinas en el extracto de Allium cepa y sus formulaciones homeopáticas (tintura madre, Allium cepa 30C y Allium cepa 200C).

Materiales y métodos: En todos los estudios, se utilizó Allium cepa tintura madre, 30C, 200C (Dr. Reckeweg). Todos los restantes productos químicos se han procurado de Sigma Aldrich. Todos los reactivos se han preparado cumpliendo los protocolos estándar.

Resultados: En el extracto preparado, así como en la tintura madre, se ha confirmado la presencia de diferentes fitoquímicos como flavonoides, alcaloides, saponinas, esteroides, hidratos de carbono y aminoácidos. Conforme a los cálculos aplicando los límites de Avogadro, las preparaciones por encima de la dilución de la 12C no deben mostrar presencia del material de origen. Cabe destacar que pese a que las formulaciones de Allium cepa 30C y 200C se consideran ultradiluciones, han dado resultados positivos para muchos fitoquímicos.

Conclusiones: Los resultados cualitativos y cuantitativos positivos también refuerzan la creciente consideración de que la homeopatía no solo es un efecto placebo, sino una medicina “inteligente” que puede funcionar a nanoescala.

Analyse phytochimique et évaluation du potentiel antioxydant de l'extrait éthanolique d'Allium cepa et des dilutions homéopathiques ultra-élevées disponibles sur le marché: étude comparative

Abstrait

Contexte: Selon les statistiques disponibles auprès de l'OMS, 80% de la population de la Terre s'appuient sur la médecine traditionnelle pour leurs besoins primaires en soins de santé et la plupart de cette thérapie implique l'utilisation d'extraits de plantes et de leurs composants actifs.

Objectif: Une analyse phytochimique qualitative a été effectuée pour confirmer la présence de divers composants comme les alcaloïdes, les graisses, les stéroides, les tanins, les flavonoïdes, les sucres, les acides aminés, les saponines dans l'extrait Allium cepa et ses formulations homéopathiques (Teinture mère, Allium cepa 30C et Allium cepa 200C).

Matériaux et Méthodes: Allium cepa MT, 30C, 200C (Dr. Reckeweg) a été utilisé pour toutes les études. Tous les autres produits chimiques ont été achetés chez Sigma Aldrich. Tous les réactifs ont été préparés selon des protocoles standard.

Résultats: La présence de divers phytochimiques comme les flavonoïdes, les alcaloïdes, les saponines, les stéroides, les glucides et les acides aminés a été confirmée dans l'extrait préparé ainsi que dans la teinture mère. Selon les calculs utilisant la limite d'Avogadro, les préparations au-dessus de la dilution 12C ne devraient pas présenter de source. Il est intéressant de noter que même si les formulations Allium cepa 30C et 200C sont considérées comme des dilutions ultra-élevées, elles ont donné un résultat positif pour de nombreux produits phytochimiques.

Conclusion: les résultats qualitatifs et quantitatifs positifs renforcent également la croyance croissante que l'homéopathie n'est pas seulement un effet placebo, mais un «médicament intelligent» qui peut fonctionner à l'échelle nanométrique.
紅洋蔥酒精提取物及市面上超高度順勢療法稀釋液之抗氧化潛能的植物化學分析及評估：對比研究

摘要

背景：據世界衛生組織的統計，地球上80%人口依賴傳統醫學以滿足基層醫療保健的需要，大多數傳統醫學都涉及植物提取物及活性成分。

目標：進行了一項定性的化學分析，以確認紅洋蔥酒精提取物及其順勢療法稀釋液中（紅洋蔥母酊、紅洋蔥30C、及紅洋蔥200C），是否含有多種成分，如生物鹼、脂肪、類固醇、丹寧酸、黃酮類物質、糖類、氨基酸及皂苷。

材料與方法：所有研究均使用（芮克偉醫生的）紅洋蔥母酊、紅洋蔥30C、及紅洋蔥200C。所有其他化學品均從西格瑪奧德里奇公司採購。所有試劑都按標準的議定方案預備。

結果：證實在提取物及母酊中可找到不同的植物化學物質，如黃酮類物質、生物鹼、皂苷、類固醇、碳水化合物和氨基酸。根據阿伏伽德羅常數的極限來計算，12C以上的稀釋應該沒有任何原材料存在。值得注意到的是，儘管紅洋蔥30C、及紅洋蔥200C被認為是超高度的稀釋液，他們仍在多項植物化學物質的分析中呈陽性結果。

結論：陽性的定性和定量結果強化了一個愈來愈普及的信念，順勢療法並不僅僅是一種安慰劑效應，而是一種「聰明的醫藥」，可能在納米的尺度下運作。