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RESEARCH

PLANT EXTRACTS TITRATION WITH POTASSIUM PERMANGANATE TO DETERMINE ANTIOXIDANT CAPACITY

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Abstract: During cellular metabolism, some unstable molecules called free radicals are produced. Free radicals are capable of binding to other molecules and cellular structures, affecting their proper functioning, such as enzymes and cell membranes. On the contrary, cells have several systems to neutralize free radicals, for example molecules such as proteins and glutathione function as antioxidants capable of buffering the harmful effects of free radicals. An imbalance between free radicals and cellular antioxidants is known as oxidative stress. Oxidative stress has been associated with multiple diseases such as hypertension, diabetes, obesity, immunodeficiencies, cancer, etc. Currently, healthy lifestyles promote adequate nutrition and incorporate foods rich in antioxidants. Unfortunately, there is no method considered the gold standard for determining the antioxidant capacity of foods or substances. The methods used to determine antioxidant capacity use free radicals such as ORAC-PE, FRAP, TRAP, ABTS and DPPH. These methods are laborious and expensive, so the objective of this work is to evaluate potassium permanganate to determine the antioxidant capacity.

Keywords: Permanganate, Potassium, Gallic acid, Antioxidant capacity, Plant extracts

INTRODUCTION

A chemical element is any substance that cannot be broken down into a simpler substance by any ordinary chemical process (Kennelly et al., 2023; Williams, 1997; Jomova et al., 2022). Those elements found inside a cell are known as bioelements (Pañuelas et al., 2019; Maret& Blower, 2022). The mainbioelements are carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur (Chesson et al., 2008). Some bioelementsjoin with other bioelements to form chemical groups, for example, the hydroxyl group is formed by oxygen and hydrogen (-OH), the sulfhydryl group isformed by sulfur and hydrogen (-SH), the amino group is formed by nitrogen and two hydrogens (-NH2), and so on (Cramet et al., 2019; Trivendi et al., 2009; Richardson et al., 2020; Prescher&Bertozzi, 2005). When chemical groups are attached to a carbon structure, the resulting compound is known as a biomolecule (Dorado et al., 2021). Based on the chemical groups present in a biomolecule, the biomolecule is classified into carbohydrates, lipids and proteins, which are the main components of all cellular structures and metabolism (Goodpaster& Sparks, 2017; Yang et al., 2019). Chemical groups are responsible for the chemical reactivity and physical-chemical characteristics of biomolecules (Yu et al., 2022). Under normal conditions, during cellular metabolism, certain unbalanced atoms or molecules are produced, for example, superoxide ($O2^{-}$), hydrogen peroxide (H2O2), and other free radicals. Those components that destabilize other molecules are known as free radicals (Andrés et al., 2023; Sies, 2017; Kehrer, 1993). The cell has several mechanisms to deal with free radicals, for

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instance, glutathione is a buffer against oxidative damage caused by reactive oxygen species such as free radicals and peroxides (Averill-Bates, 2023; Bielli et al., 2015). An imbalance between free radicals and cellular antioxidants is known as oxidative stress (Pisoschi, 2015; Sies, 2015). Oxidative stress has been associated with multiple diseases such as hypertension, diabetes, obesity, immunodeficiencies, cancer, etc. (Forman & Zhang, 2021; Jones, 2008; Hajam et al., 2022). Currently, healthy lifestyles promote adequate nutrition and incorporate foods rich in antioxidants (Bojarczuk&Dzitkowska-Zabielska, 2022; Wang et al., 2017). Unfortunately, there is no method that is considered the gold standard for determining the antioxidant capacity of foods or substances (Pinchuk, et al., 2012). The methods used to determine antioxidant capacity employ free radicals such as ORAC-PE, FRAP, TRAP, ABTS and DPPH (Fernández-Pachón et al., 2005; Rumpf, et al., 2023; Munteanu &Apetrei, 2021; Huang et al., 2005). These methods are laborious and expensive, so the objective of this work was to evaluate potassium permanganate to determine the antioxidant capacity.

MATERIALS AND METHODS

Neem extracts

Fresh samples of *Azadirachta indica* (Neem) were used to prepare extracts from various parts of the plant. The samples collected were 800 g of neem leaves, 500 g of neem bark, 500 g of neem fruit, 200 g of neem seed, and 200 g of neem flower. They were collected during the months of March and April in the city of Lerdo (Durango, Mexico) from a local vivarium. After being collected, the samples were rinsed several times with tap water, separated, and allowed to dry at room temperature. Once dry, thesamples were ground with a manual mill, and mixed in a 1:10 solution with hot distilled water at 60° C for 1 h, then filtered with a Whatman number 40 filter and left to dry in a hot air oven at a temperature of 40° C for a period of 7 days.

Phytochemical tests

These tests were qualitative to identify the main chemical groups of organic compounds present in plant extracts. The principle of these tests is based on chemical reactions between the functional chemical groups of organic compounds present in plant extracts and chemical reagents that lead to the formation of precipitates or colored substances. The following determinations were madein accordance with methodology published elsewhere (Barboza-Herrera et al., 2021): 1) Alkaloids (Dragendorff and Mayer'smethod); 2) Aldehydes (Tollens' method); 3) Carbohydrates (Brady'smethod); 4) Flavonoids (Magnesium method); 5) Sterols (Liebermann-Burchard's method); 6) Tannins (Indirect precipitation method); and 7) Terpenoids (Chloroform method).

Potassium permanganate titration

A 0.25 M KMnO4 solution was standardized with 134 mgsodium oxalate. The following formula was used to calculate the Normality concentration.

$$N = nM$$

Where N refers to Normality, n refers to the quantity of equivalents and M refers to Molarity. the Normal concentration of the KMnO4 solution was 1.25 according to the reduction of Mn+7 to Mn+2 with n=5. Sodium oxalate is a primary standard *i.e.* a reagent which can be easily weighed, and is so pure that its weight is truly representative of the number of moles of substance contained. The oxidation-reduction reaction of KMnO4 and sodium oxalate in an acidic medium is as follows:

$$2MnO_4^- + 5C_2O_4^{-2} + 16\,H^+ \rightarrow 10CO_2 + 2Mn^{+2} + 8H_20$$

The standardization of the KMnO4 solution considered the following formula

$$KMnO4\ Normality = \frac{\frac{(mg\ primary\ standard)}{(equivalenst\ of\ primary\ standard)}}{\frac{(Molecular\ weight\ of\ primary\ standard)}{(mL\ of\ KMnO4\ )}}$$

The standardized KMnO4 solution was used to titrate 50 mL of aqueous solutions of neem extracts at a concentration of 200 mg/L. These solutions were prepared in 3N H2SO4. The neem extracts used in this study were leaves, bark, fruit, seed and flower. After titration withpotassium permanganate, the Normal concentration was calculated with the following formula

$$V_{KMnO_4} C_{KMnO_4} = V_{Sample}\, C_{Sample}$$

Where the volumes (V) of KMnO4 and the plant extract solution samples were measured in mL, and their concentrations in Normality. Once the samples were titrated and the normal concentrations were calculated, the redox equivalents were determined considering that the definition of normality is the number of equivalents of a solute divided by the volume of the solution in liters according to the following formula

$$n = \text{NL}$$

Where n refers to the quantity of equivalents, N refers to the Normal concentration and L to the volume of the solution in liters. Finally, the number of redox equivalents per gram of dry extract was calculated with the following formula

$$Redox\ equivalents\ per\ gram = \frac{n}{grams\ of\ extract}$$

Another alternative method to calculate antioxidant capacity was gallic acid equivalents. The standardized KMnO4 solution was used to titrate 50 mL of neem extract solutions at a concentration of 200 mg/L. The results were interpolated against a standard curve of a serial dilution of gallic acid (0.2, 0.4, 0.6, 0.8, 1.0, 1.2, 1.4, 1.6, 1.8 and 2.0

mM), which was titrated with the standardized KMnO4 solution. The statistical analysis was descriptive through mean values of the variables under study; and a linear regression to calculate the gallic acid equivalents.

RESULTS AND DISCUSSION

The qualitative phytochemical tests of the aqueous extracts of neem leaves, bark, fruit, seed, and flower allowed identification of the presence of carbohydrates, flavonoids, sterols, tannins and terpenes as shown in Table 1.

Table 1. Phytochemical tests of the Neem extracts.

Phytochemical test	Neem extracts				
	Leaves	Bark	Fruit	Seed	Flower
Alkaloids	-	-	-	-	-
Aldehydes	-	-	-	-	-
Carbohydrates	+	+	+	+	+
Flavonoids	+	+	+	+	+
Sterols	-	-	-	+	-
Tannins	+	+	-	+	-
Terpenoids	+	+	+	-	+

+ positive, - negative.

The analysis of antioxidant capacity showed a similar trend between the results obtained as redox equivalents and gallic acid equivalents as appears in Table 2. The volume of the titrant (KMnO4) and the concentration of gallic acid were used to perform a linear regression (r=0.96). The neem flower extract exhibited the highest antioxidant capacity, followed by the fruit and bark extracts. The neem leaves and bark extracts exhibited the lowest antioxidant capacity.

Table 2. Analysis of antioxidant capacity by potassium permanganate titration.

Neem extract	Normality of titrated solution	mEq in the titrated solution	Redox equivalents per gram of dry extract	Gallic acid equivalents (mM)
Leaves	0.0025	0.125	12.5	0.1135
Bark	0.0025	0.125	12.5	0.1135
Fruit	0.005	0.25	25	0.2113
Seed	0.005	0.25	25	0.2113
Flower	0.0075	0.375	37.5	0.3092

The KMnO4 is a chemical compound formed by potassium ions (K^+) and permanganate (MnO^{-4}). In addition, it is a strong oxidizing agent with an intense violet color, so it works as an auto indicator (Eteiwi et al., 2015). A violet solution of MnO^{-4} is easily reduced to a colorless solution of Mn^{+2} (Zhang et al., 2014). It has a very high standard reduction potential; therefore, it is a very strong oxidant and can oxidize any compound with a lower standard reduction potential (Homolak et al., 2021). Normality in chemistry is a unit of concentration of a solution and is defined as the relationship among the equivalents of a substance and the liters of that solution. Normality is a measure of reactive capacity. An equivalent refers to the amount of the solute that will react with a known amount of another reactant. Normality is frequently used for oxidation-reduction reactions in which electrons are transferred from one reactant to another. In this work, the end point of the titration of the tested plant extracts was indicated by the appearance of a permanent light pink color due to a slight excess of potassium permanganate solution. Among the limitations of this work, it is worth mentioning that some plant extracts are rich in pigments that can mask the end point of the permanganate titration. Additionally, some compounds in plant extracts can form brown magnesium oxide, which could mask the end point of the titration. Consequently, plant extracts should be selected with caution for titration with this method.

CONCLUSION

Extracts from neem leaves, bark, fruit, seed, and flowers were prepared and analyzed by potassium permanganate titration, and the results were expressed as redox equivalents and gallic acid equivalents. The results of both types of equivalents showed a similar trend. Thus, in this research work, the usefulness of KMnO4 was

demonstrated to determine the antioxidant capacity of plants extracts.

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