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Article

Alkaloids Detection in Commonly Found Medicinal Plants with Marquis Reagent

Daniel Alejandro Ocampo-Bustos[1] and María Elena Cano-Ruiz[1]
[1] Tecnológico de Monterrey High School, Cuernavaca, Mexico.

SUMMARY

Alkaloids are a class of nitrogenous organic compounds of plant origin that may have important physiological actions on humans. They include many drugs and poisons, but some alkaloids in low doses have health benefits as well. Traditional medicinal plants may contain alkaloids as active ingredients, but this is not well-understood. The Marquis reagent exists as a simple qualitative colorimetric method to determine the presence of alkaloids in medicinal plants. The Marquis reagent test was assayed in medicinal plants by first optimizing the formulation of the reagent using poppy seeds and lavender as the positive and negative controls. Then using the optimized formulation of Marquis reagent in the extracts of 11 medicinal plants with known claims of health benefits. Four medicinal plants tested positive for alkaloids, including a relaxing herbal mix (*Tilia cordata*, *Valeriana officinalis*, *Passiflora incarnata*, and *Melissa officinalis*), *Turnera diffusa*, *Brickellia cavanillesii*, and *Verbascum thapsus*. These findings demonstrate the value of the Marquis reagent test to provide a rapid and simple method for screening for potentially medicinal alkaloids of natural origin.

INTRODUCTION

Alkaloids are a class of nitrogenous organic compounds, usually with aromatic rings, that are widely known for their effects on organisms. These effects are put to use in many kinds of medications, such as analgesics, relaxants, and even antiarrhythmics. They are also naturally present in some plants like *Papaver somniferum*, or poppy, from which several opium alkaloids are extracted; for example, morphine is the most abundant alkaloid in the poppy plant (1, 2).

Alkaloids, in their vast majority, can cause addiction problems or toxicity when consumed by humans, which is why most alkaloids are either prohibited, restricted, or, preferably, controlled by governments globally for availability only with a medical prescription (3). On the other hand, in very low doses, alkaloids seem to have therapeutic effects on certain conditions, such as different kinds of pain, stress, hyperactivity, or nervous system-related illnesses (4).

Phytotherapy is the use of medicinal plants or extracts of natural origin used to prevent and treat different diseases, which is also called folk medicine. In Mexico, this represents a system rooted in the deepest part of the cosmovision and identity of social groups. Many of the medicinal plants have their healing properties known by empirical use through time, but these medicinal plants may contain active ingredients with tested pharmacological properties. One possibility is that some of the active ingredients in medicinal plants belong to the group of alkaloids, which can be determined by a colorimetric chemical reaction with the Marquis reagent. The reagent is dripped onto the substance being tested, and if an alkaloid is present, a color change appears (5). The Marquis reagent is traditionally composed of a mixture of formaldehyde and concentrated sulfuric acid.

Originally, the Marquis reagent was used for testing many different alkaloids, and the results from those studies were the base for developing the color scales that are used as a reference to determine the specific alkaloid that is present in a solution (5, 6). The Marquis reagent can give different color changes to hundreds of drugs (7). Purple-dark or blue-dark colors are observed with methylenedioxylphenyl groups in alkaloids, which is present in methylenedioxymethamphetamine (i.e., MDMA, ecstasy); orange is observed with amphetamines; blue-black to yellow colors are observed with methylenedioxy-substituted analogs; and red-violet is observed with methylenedioxyphenyl amphetamine-type compounds (8, 9).

In this study, a possible explanation for the medicinal properties of some plants was elicited by determining the presence of alkaloids in 11 common medicinal plants that are found in local markets of Mexico. We hypothesized that plants with relaxing effects or claims of better sleep would contain alkaloids, as many medications available in pharmacies for these purposes (e.g., clonazepam) are alkaloids. Using the Marquis reagent qualitative colorimetric test, we tested the 11 common medicinal plants for the presence of alkaloids. Four medicinal plants tested positive for alkaloids indicating the value of the Marquis reagent test as a rapid and economical method for screening for potentially medicinal alkaloids of natural origin.

RESULTS

Marquis reagent is a colorimetric test commonly used for the detection of alkaloids. To obtain the best preparation for the Marquis reagent, we tested several formulations in triplicate varying the concentrations and amount of formaldehyde, acetic acid, and sulfuric acid (10).

Comparing the different color tones that the mixture took

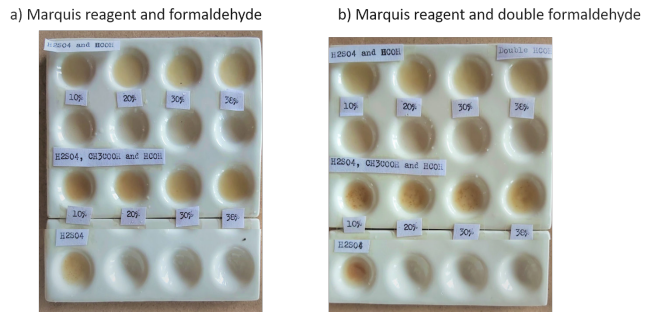

Figure 1: Optimization of Marquis reagent preparation using poppy seed macerate as a positive control. A) (left). Poppy seed macerate was tested after 45 s with the different preparations and concentrations of the Marquis reagent. First line, no acetic acid added; second line, no samples; third line, acetic acid added; bottom-left sample, control sample with sulfuric acid only. First and third lines from left to right: 10%, 20%, 30%, and 38% of formaldehyde. All preparations include 1 mL concentrated sulfuric acid and 0.1 mL formaldehyde. B) (right). Poppy seed macerate was tested after 45 s with the different preparations and concentrations of the Marquis reagent (double formaldehyde). First line, no acetic acid added; second line, no samples; third line, acetic acid added; bottom-left sample, control sample with sulfuric acid only. First and third lines from left to right: 10%, 20%, 30%, and 38% of formaldehyde. All preparations include concentrated 1 mL sulfuric acid and 0.2 mL formaldehyde.

with the poppy seed macerate when different formulations of the Marquis reagent were added, we determined that there was an alkaloid present (morphine) in the poppy seed macerate when the color changed from white to different tones of light orange-brown. All poppy seed samples originated from the same macerate, so the different tones observed are resultant of the different reagent formulations rather than variances in the samples (**Figure 1A & B**).

Reaction time must be measured during the colorimetric tests because sulfuric acid in the reagent will continue reacting with organic compounds, producing a dark color; therefore, the test must be completed in less than 60 seconds. The most sensitive formulation of the Marquis reagent for color development contained 10% formaldehyde in 99% acetic acid and 98% sulfuric acid (**Figure 1A**). We selected and used this formulation for determining the presence of alkaloids in the 11 common Mexican medicinal plants.

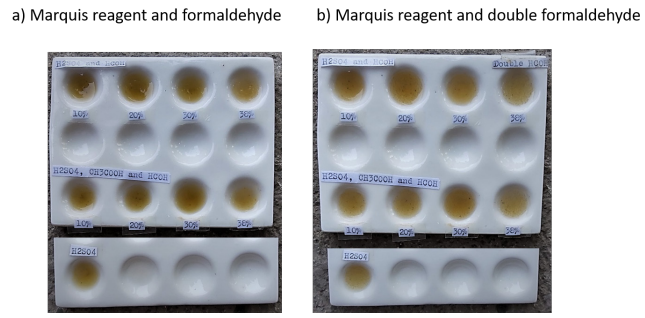

Figure 2: Optimization of Marquis reagent preparation using lavender macerate as a negative control. A) (left). Dry lavender macerate after 45 s with the different preparations and concentrations of the Marquis reagent. First line: no acetic acid added, second line, no samples; third line: acetic acid added; bottom left sample, control sample with sulfuric acid only. First and third lines from left to right: 10%, 20%, 30%, and 38% of formaldehyde. All preparations include 1 mL concentrated sulfuric acid and 0.1 mL formaldehyde. B) (right). Dry lavender macerate after 45 s with the different preparations and concentrations of the Marquis reagent (double formaldehyde). First line: no acetic acid added; second line, no samples; third line: acetic acid added; bottom left sample, control sample with sulfuric acid only. First and third lines from left to right: 10%, 20%, 30%, and 38% of formaldehyde. All preparations include 1 mL concentrated sulfuric acid and 0.2 mL formaldehyde.

Figure 3: Representative images of tea preparations. From left to right, tea preparations of *Tilia cordata*, *Turnera diffusa*, and the relaxing herb mix, using 2 g of dried plant in 100 mL of water at 90°C.

Dry lavender was used as a negative control in this experiment because it does not contain alkaloids (11). The Marquis reagent did not present a change of color with lavender with any of the triplicate tests, where the color of the macerate extract was dark-greenish-brown and remained the same color in all Marquis formulation tests (**Figure 2A & B**).

We prepared teas from 11 common medicinal plants by three different procedures: a standard preparation using 2 g dried herbs in 100 mL of hot water, a concentrated preparation using 2 g dried herbs in 50 mL of hot water, and a macerated preparation with the 2 g of dried herbs blended in 100 mL of hot water (**Figure 3**). We added equal volumes of each tea preparation to porcelain trays (**Figures 4A, 5A, and 6A**). A sulfuric acid only control was used to control for any color changes due to the sulfuric acid and not the Marquis reagent with the macerated preparations (**Figure 7A**). We used the macerated preparation for the sulfuric acid control because,

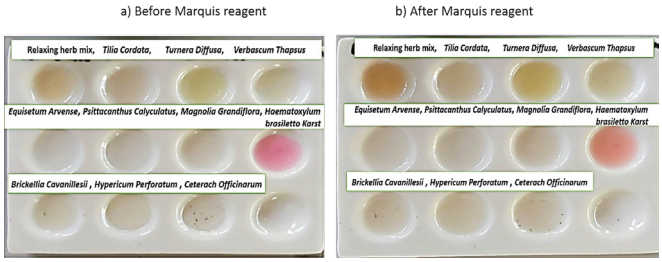

Figure 4: Marquis reagent test for medicinal plants using a standard tea preparation. A) (left). Tea samples of each of the 11 medicinal plants before the Marquis reagent test. Standard preparation, with 2 g of tea in 100 mL of water at 95°C and letting it rest for 5 min. B) (right). Tea samples of each of the 11 medicinal plants after the Marquis reagent test. Positive change in color was observed for the relaxing herbs mix (first line, position one), *Turnera diffusa* (first line, position three), and *Verbascum thapsus* (first line, position four).

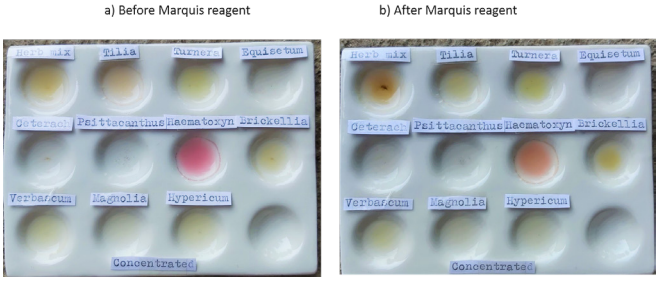

Figure 5: Marquis reagent test for medicinal plants using a concentrated tea preparation. A) (left). Tea samples of each of the 11 medicinal plants before the Marquis reagent test. Concentrated preparation, with 2 g of tea in 50 mL of water at 95°C and letting it rest for 5 min. B) (right). Tea samples of each of the 11 medicinal plants after the Marquis reagent test. Positive change in color was observed for the relaxing herbs mix (first line, position one), *Tilia cordata* (first line, position two), *Turnera diffusa* (first line, position three), *Haematoxylum brasiletto karst* (second line, position three), Brickellia cavanillesii (second line, position four) and *Verbascum thapsus* (third line, position one).

even though they were strained, they could have had very small organic residues from the leaves of the plants which may react with the sulfuric acid in the reagent and change the color of the test. Therefore, with this control, we were certain that the color obtained in the test was not due to the sulfuric acid reacting with confounding organic residues.

For color development, we added the Marquis reagent to all herbal samples (standard, concentrated, and macerated) and for sulfuric acid control, we added sulfuric acid instead of Marquis reagent (**Figures 4B, 5B, 6B, and 7B**). After the reaction time passed, any color changes were observed (**Figures 4–7**). After comparing with the color development in the sulfuric acid control tray, any color change in a sample after Marquis reactant addition was considered a positive result for the presence of alkaloids, independent of the tone or intensity of the color. Different preparations (standard, concentrated, and macerated) gave similar results for all

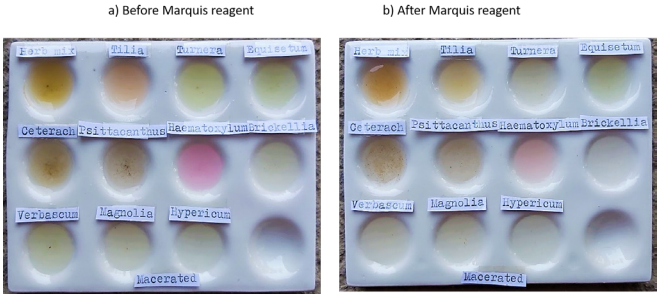

Figure 6: Marquis reagent test for medicinal plants using a macerated tea preparation. A) (left). Tea samples of each of the 11 medicinal plants before the Marquis reagent test. Macerated preparation with 2 g of tea in 100 mL of water at 95°C was added to the blender at medium speed for 30 s and allowed to rest for 60 s. B) (right). Tea samples of each of the 11 medicinal plants after the Marquis reagent test. Positive color change was observed for the relaxing herbs mix (first line, position one), *Tilia cordata* (first line, position two), *Turnera diffusa* (first line, position three), *Haematoxylum brasiletto karst* (second line, position three), and *Verbascum thapsus* (third line, position one).

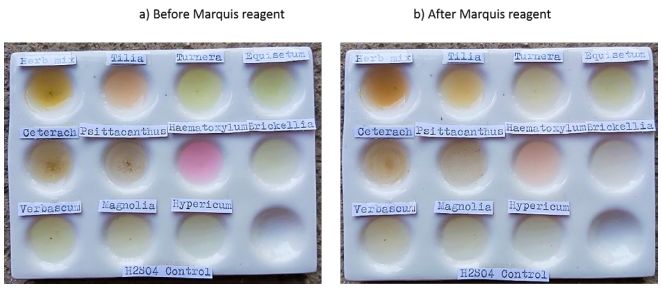

Figure 7: Control test for medicinal plants using a macerated tea preparation and sulfuric acid. A) (left). Tea samples of each of the 11 medicinal plants before adding sulfuric acid. Macerated preparation with 2 g of tea in 100 mL of water at 95°C was added to the blender at medium speed for 30 s and allowed to rest for 60 s. B) (right). Tea samples of each of the 11 medicinal plants after adding sulfuric acid. Positive color change was observed for the relaxing herbs mix (first line, position one), *Tilia cordata* (first line, position two), *Turnera diffusa* (first line, position three), *Haematoxylum brasiletto karst* (second line, position three), and *Verbascum thapsus* (third line, position one).

herbal samples tested. The results from the color changes and possible alkaloids present in the 11 medicinal plants assayed were recorded (**Table 1**).

After adding the Marquis reagent to the standard preparation of the medicinal plants, the relaxing herbs mix changed color from light orange to dark-reddish-brown, *T. diffusa* changed color from light green to orange-yellow, and *V. thapsus* had a color change from pale green to light orange-brown (**Figure 4A & B**). The concentrated preparation after adding the Marquis reagent also had color changes in the relaxing herbs mix from light orange to dark-reddish-brown, in *T. cordata* from pink-orange to yellow-orange, in *T. diffusa*

Plant	Color before test	Color after test	Possible Alkaloid present
Tilia cordata	Pink-orange	Orange (In concentrated and macerated tests only)	Possible, although the color change may be due to a reaction with sulfuric acid only
Turnera diffusa	Light green	Orange-yellowish	YES
Equisetum arvense	Transparent	Transparent	-
Ceterach officinarum	Transparent	Transparent	-
Psittacanthus calyculatus	Transparent	Transparent	-
Haematoxylum brasiletto karst	Pink	Pink-orange (In the concentrated and macerated tests only)	None, the color change was due to the sulfuric acid in the reagent.
Brickellia cavanillesii	Very light pale greenish-yellow	Light orange (in the concentrated)	YES
Verbascum thapsus	Pale green	Light orange-brown	YES
Magnolia grandiflora	Transparent	Transparent	-
Hypericum perforatum	Transparent	Transparent	-
Relaxing herbs mix *(Tilia cordata, Valeriana officinalis, Passiflora incarnata,* and *Melissa officinalis)*	Light orange	Dark reddish-brown	YES

Table 1: Results from the Marquis reagent colorimetric tests with 11 medicinal plant tea extracts.

from light green to yellow, in *H. brasiletto karst* from pink to pink-orange, in *B. cavanillesii* from a light green-yellow to light orange, and *V. thapsus* from pale green to a light orange-brown (**Figure 5A & B**). The macerated preparation had also color changes after the addition of Marquis reagent in the samples of the relaxing herbs mix, *T. cordata*, *T. diffusa*, *H. brasiletto karst*, and *V. thapsus* very similar to the color changes in the concentrated preparations. (**Figure 6A & B**). Finally, the sulfuric acid control had color changes in the relaxing herbs mix (from light orange to dark orange), *T. cordata* (from pink-orange to yellow-orange), *T. diffusa* (from light green to light brown), *H. brasiletto karst* (from pink to pink-orange), *B. cavanillesii* (light green-yellow to a light brown) and *V. thapsus* (from pale green to pale orange) (**Figure 7A & B**), but these color changes with only sulfuric acid were different from the color changes observed with the Marquis reagent for the relaxing herbs mix, T. diffussa, B. cavanillessii, and *V. thapsus*, (**Figures 4, 5 and 6A &B**) indicating the presence of alkaloids for these four plants.

DISCUSSION

In this study, the Marquis reagent tested with the poppy seeds macerate (white color) was the positive control due to its morphine content to determine which formulation of Marquis reagent was more sensitive (**Figure 1**). The formulation composed of 98% sulfuric acid, 99% acetic acid, and 10% formaldehyde gave the strongest color change and was used for testing the medicinal plants for the presence of alkaloids (**Figure 1A**). We anticipated that increasing the concentration of formaldehyde would result in darker color development (10). However, increasing formaldehyde concentration did not increase color intensity, and these results were consistent for the three trials. Standard Marquis preparations use 40% formaldehyde for alkaloid detection (6, 8, 10). A possible explanation for our results could be that the macerate preparations extracted had a lower concentration of alkaloid and that the amount of formaldehyde in the 10% formulation was sufficient to complex with the alkaloid present. Therefore, no more color changes would result with the addition of formaldehyde, as no more alkaloid-formaldehyde complexes would form at a higher concentration of formaldehyde. Furthermore, doubling the volume of formaldehyde used did not improve color development or intensity, and some precipitation was observed (**Figure 1B**). The addition of acetic acid seemed to help the formation of initial alkaloid-formaldehyde complexes, as preparations with acetic acid gave more intense colors than those without it (**Figure 1A**). Possibly, the increased amount of acid increased the solubility of formaldehyde or the rate of color development. Some Marquis reagents use acetic acid for dissolving the formaldehyde before adding sulfuric acid (10, 12, 13). Other preparations use a lower concentration of formaldehyde in the final Marquis reagent, such as the 10% formaldehyde Marquis reagent formulation that was the most sensitive in the positive control essays (12, 13).

The lavender macerate, which does not contain alkaloids, did not produce a color change; it remained dark greenish-brown following the addition of the Marquis reagent formulations and the sulfuric acid control. Therefore, we concluded that the Marquis reagent could be used as a specific test for the presence of alkaloids in medicinal plants.

Results from the tea extracts varied depending on the preparation method, but in general, the relaxing herbs mix, *T. diffusa*, *V. thapsus*, and *B. cavanillesii* all showed a color change indicating that all four contain alkaloids. Additionally, the concentration of alkaloids was higher in the relaxing herbs mix and *T. diffusa* samples as they had more robust color changes compared to *V. thapsus* and *B. cavanillesii*.

For the standard preparation method, the relaxing herb mix, *T. diffusa*, and *V. thapsus* samples changed colors. For the concentrated preparation method, the relaxing herbs mix, *T. diffusa*, *V. thapsus*, *B. cavanillesii*, *T. cordata*, and *H. brasiletto karst* samples changed colors. For the macerated preparation method, the relaxing herbs mix, *T. diffusa*, *T. cordata*, *H. brasiletto karst*, and *V. thapsus* samples changed colors (**Table 1**).

These color changes may support that all of these medicinal plants contained alkaloids, but for more reliable results, we compared the color changes with the samples exposed to only sulfuric acid in the control and no Marquis reagent (**Figure 7A & B**). We concluded that the relaxing herbs mix, *T. diffusa*, and *V. thapsus* contained alkaloids because the results were positive in the three preparations, as well as the sulfuric acid control, but the sulfuric acid control tests produced different colors than those with Marquis reagent, so the color change was not because of the sulfuric acid in the Marquis reagent but a specific Marquis reagent reaction with alkaloids.

Our results agree with previous studies that found alkaloids in the plants that tested positive in our study. One of the plants in the relaxing herbal mix, *V. officinalis*, contains two known alkaloids: catinine and valerian (14). One study reported the presence of the alkaloid caffeine in leaves and stems of *T. diffusa* (15). Although it is known to contain phenolic and saponins compounds and studies for alkaloids content are incomplete for this plant, *V. thapsus* tested positive for alkaloids (16). Nevertheless, *V. thapsus* belongs to the Scrophulariaceae family, which is a source of a variety of chemical constituents like saponins, monoterpene glycosides, iridoids, phenylethanoid glycosides, neolignan glycosides, flavonoids, steroids, phenolic acids, fatty acids, and spermine alkaloids that may explain its positive test (17). It is likely that *B. cavanillesii* also contains alkaloids because, although it produced no color change in the standard and macerated preparations, it did produce a color change in the concentrated preparation, which used half the volume of water as the other two methods. This difference in results along preparations methods indicates that concentration was an important variable for detecting color changes. *B. cavanillesii* has been shown to contain terpenes, terpene

derivatives, esters, ketones, aldehydes, and phenol-derived aromatic compounds (18). Also, *B. cavanillesii* belongs to the Asteraceae family, which is a source of pyrrolizidine alkaloids and may explain its positive result (19). Finally, *T. cordata* and *H. brasiletto karst* produced color changes in both the concentrated and macerated preparations tests; however, they likely do not contain alkaloids because the samples developed the same color when tested with the sulfuric acid control. Therefore, other compounds different than alkaloids were responsible for the color change observed with Marquis reagent. Most studies of color development of Marquis reagent have been done with known alkaloid drugs, but few have studied other related naturally-occurring compounds, such as terpenes and substituted allylbenzenes like safrole, which is used as a precursor for amphetamine-type drugs (6, 8, 9, 12, 20-22).

The method we used was a qualitative colorimetric method, performed with tea extracts and not pure alkaloids, so it is very difficult to compare colors obtained in the experiment with the standard Munsell Chart for colorimetry typically used for the determination of specific alkaloids in drugs and other known compounds (6). Therefore, it was difficult to elucidate the types of alkaloids present in medicinal plants. Additionally, the intensity of the color was much less than what is standard due to the lack of purity and the low concentrations of alkaloids present in the medicinal plants. Studies show that Marquis reagent produces pale colors when safrole concentration in essential oils is less than 15%, but it gives a dark blue-violet color in essential oils with safrole concentrations greater than 80% and with pure safrole (12). We believe that the concentration of alkaloids in the sample is important for both positive detection and color development.

Additionally, if two or more alkaloids are present in a certain plant, two possible scenarios arise. First, if one alkaloid is present in a higher concentration than others, then it would not substantially modify the final color; the mixture of the other colors with the one at the highest concentration would not be enough to change the result. Alternatively, if all the alkaloids are present at similar concentrations, then the resulting color would be a mixture of all the alkaloids present. This experiment only considered water-soluble alkaloids as they were the ones extracted by the hot water brewing method used in the tea preparations. Alkaloids that are not water-soluble were not detected, and they were not considered in this experiment. The medicinal plants that tested positive for alkaloids have the following health benefit claims, according to the seller from the local Mexican market: *T. diffusa*, nervous system alterations relief; *B. cavanillesii*, alleviating digestive and bile problems; *V. thapsus*, suppressing cough; and the relaxing herb mix, for relaxation.

To identify specific alkaloids in the preparations of medicinal plants that tested positive for alkaloids and to determine their concentration, more efficient extraction methods, and more specialized analytic instruments (i.e., high-performance liquid chromatography (HPLC) or gas chromatography-mass spectrometry (GC-MS) will be needed. However, the Marquis reagent could be used as an economical and rapid screening method for the detection of alkaloids in medicinal plants.

MATERIALS AND METHODS

Qualitative visual colorimetric analyses with the different Marquis reagent formulations were done to determine the presence of the alkaloids in the poppy seeds and the medicinal plants. If a color change was observed, the test was considered positive for the presence of alkaloids, independently of the color obtained.

Observation of color changes is subject since the interpretation of color may vary from person-to-person. To diminish differences in color interpretations, we took photos before and after the addition of the reagent with the best light possible to ensure color changes were only affected by the chemical reactions in the tests. We registered qualitatively positive tests when a color change occurred, independently of the color observed and its intensity.

Marquis Reagent Preparation

Two preparation methods of the Marquis reagent were tested, differing from the traditional Marquis formulation, by addition of acetic acid and by variation of formaldehyde concentration. These methods were included based on a study that stated with some alkaloids, specifically aspirin, the preparations showed reliable results (10). The procedure from this previous study was slightly modified to generate two preparations with or without acetic acid and using different concentrations of formaldehyde were tested for the Marquis reagent as described below.

Preparation A consisted of four formulations of Marquis reagent using 1 mL of 98% sulfuric acid and 0.1 mL of varying formaldehyde concentrations of 10%, 20%, 30%, or 38%. Then, the other four formulations of Marquis reagent were composed of 1 mL of 98% sulfuric acid and 0.2 mL of varying formaldehyde concentrations of 10%, 20%, 30%, or 38%. Preparation B consisted of four formulations of Marquis reagent using 0.1 mL of 10%, 20%, 30%, or 38% formaldehyde in 1 mL 99% acetic acid solution and 1 mL of 98% sulfuric acid. Then, the other four samples of Marquis reagent were composed of 0.2 mL of 10%, 20%, 30%, or 38% formaldehyde in 1 mL 99% acetic acid solution and 1 mL of 98% sulfuric acid.

Marquis Reagent Assay

After all formulations were prepared, a macerate of 30 mL of water and 15 g of poppy seeds was prepared for the positive control (morphine alkaloid present), and another macerate of 30 mL of water and 15 g of dry lavender was prepared for the negative control (no alkaloid present). Each macerate was prepared by blending the seeds or dry plant with water at medium speed for 30 s. The macerates were then filtered to mitigate any organic residues of the seeds or stems, which could be burned by the sulfuric acid in the Marquis reagent

and modify the color.

Preparations A and B were tested on both the poppy seed and lavender macerates to determine the best formulation of the Marquis reagent, using 1 mL of macerate and 2 drops of the Marquis reagent. The macerates and reagent were allowed to react for no more than 60 s to decrease sulfuric acid reacting with any remaining residue from the poppy seeds or the lavender and modifying the color. A reaction time of 45 s was considered to be sufficient because it was not so little time that the sample would not be able to change color and neither so much time that it would become dark. Photographs could also be taken within this 45 s. All tests were performed three times.

Medicinal Plants Extraction and Testing

Eleven medicinal plants from a local Mexican market were assayed for the presence of alkaloids. These plants, by scientific name and their use according to the seller, were the following: *T. cordata* (relaxing), *T. diffusa* (nervous system alterations), *E. arvense* (kidney diseases), *C. officinarum* (kidney function), P. calyculatus (kidney stones), *H. brasiletto karst* (blood circulation), *B. cavanillesii* (digestive and bile problems), *V. thapsus* (coughing), *M. grandiflora* (heart disease), *H. perforatum* (cicatrization), and a relaxing herb mix (improve relaxation) of *T. cordata*, *V. officinalis*, *P. incarnata* and *M. officinalis*.

Three different preparations were tested using the preparation B Marquis reagent composed of 10% formaldehyde in acetic acid, and 0.1 mL of this preparation was combined with 1 mL of concentrated sulfuric acid. All tests were performed three times.

The standard preparation consisted of steeping 2 ± 0.2 g of each of the dried or fresh plants in a beaker with 100 mL of water at 90°C for 5 min. The concentrated preparation consisted of steeping 2 ± 0.2 g of each of the dried or fresh plants in a beaker with 50 mL of water at 90°C for 5 min. The macerated preparation consisted of steeping 2 ± 0.2 g of each of the dried or fresh plants in a beaker with 100 mL of water at 90°C. For all preparations, 1 mL of each infusion was added to a porcelain tray. Then, 2 drops of the Marquis reagent were added, a reaction was allowed to occur for 45 s, and any color changes were recorded.

A final preparation consisted of reacting a sulfuric acid control solution with the 11 medicinal plant macerated extracts, using only sulfuric acid instead of the Marquis reagent. Using a similar procedure, 1 mL of macerate from each infusion was added to the porcelain tray, 2 drops of sulfuric acid were added to each sample, and any color development was observed within 45 s. This reaction was performed to compare color changes due to sulfuric acid only.

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