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DOES CASCARA SAGRADA CONTAIN A TANNIN?*

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As the official drug, Cascara Sagrada, the last four revisions of the United States Pharmacopœia have recognized the dried bark of the trunk and branches of *Rhamnus Purshiana*, DeCandolle.

Except for use as a cathartic by the Indians of the Pacific slope of North America, this bark was not employed until about fifty years ago, at which time its purgative effects were brought to the attention of the medical profession.

While the history of the introduction of cascara sagrada, the botany of the drug and all branches of its pharmacology have been dealt with by many contributors to this knowledge, reference will be limited to such parts of the literature of this and other drugs as shall seem to be needed for a relevant report on the question under consideration.

As mentioned by Warren (*JOUR. A. PH. A.*, p. 254, (1924)) although this drug has been the subject of research by many skilful analysts its chemistry is still obscure and "the subject is so full of interrogation points that no one can to-day assert with assurance a statement of the exact chemical constituents of this drug."

That is true not only as regards the important constituents of cascara but equally the case with respect to the medicinally unimportant principles, as for example "tannin," which is listed as a constituent of ranking importance by some text and reference books, while other books do not mention tannin as present in this drug.

In the hope of removing one of the aforesaid interrogation points by determining the presence or absence of tannin, this review of the literature and examination of the bark were undertaken.

It is also true that, while much has been written on cascara sagrada, the matter of tannin as a constituent has had but casual consideration, as will be made evident by bringing the comparatively few and very meagre references together.

That such has been the case is not at all strange, as cascara bark would scarcely be suspected of containing a tannin if decision had to depend on the recognition of an astringent taste, for bitterness is the continuous impression from the first

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effect to the final passing of taste. And here may be noted the fact that the barks, berries and other parts of plants of the family *Rhamnaceæ* have found no use in leather making, except to a limited extent as stains for the already tanned skin.

There has long been an understanding that cascara sagrada should be collected at least one year before being used, and the tenth revision of the U. S. P. specifically directs for the first time that this requirement be observed. This detail is mentioned because there is reference to age of material used by certain workers who will be quoted in the course of this article.

Long before 1877, when this drug was made known to the medical profession, the similar bark of a related Old World species, *Rhamnus frangula*, then generally called buckthorn bark, but later "frangula," was to some extent used as a purgative.

As early as 1849, Biswanger (*Buchner's Repert. für Pharm.*) reported the bark of *Rhamnus frangula* to contain a yellow coloring principle, an ether-soluble amorphous resin, one or more alcohol-soluble resins, a bitter substance of resinous nature to which the purgative property of the bark was attributed, sugar, gum, tannin, plant acids, extractive, etc.

Whether the bark of *Rhamnus frangula* was in some instances substituted for that of *Rhamnus Purshiana*, or whether some of the early impressions regarding the chemistry of cascara sagrada were influenced by existing opinions and beliefs pertaining to frangula bark, there seems to be in some of the early literature a tendency to ascribe to cascara sagrada every virtue if not every constituent known for frangula.

In 1878, Prescott published in "New Preparations" the first analysis of cascara sagrada. His results were reprinted in full in the April number of the *Am. J. of Pharm.* for 1879. He reported an examination which embraced a study of the structure of the bark and of its chemical constituents; but he made no reference to the scheme of analysis that he followed, nor of any special procedure for the isolation of the nine substances which he reported. Among these he mentioned "a tannic acid, giving brownish green color with ferric salts."

Though Prescott gave no further outline of his recognition of this particular constituent, it is fair to presume that he followed the system of analysis suggested in his book "Outlines of Proximate Analysis," which had been in use by his own classes since 1874, or the methods given in his "Organic Analysis," which was published in 1887. Both of which books mention the methods of identifying "tannins" which are given in the general scheme of plant analysis suggested by Parson, Dragendorff and others whom Prescott followed, as acknowledged in his prefaces to these volumes.

While taste does not indicate the presence of tannin, a drop of ferric chloride solution put on any section of cascara sagrada and allowed to dry produces a dark green or blackish color which is likely to lead to the belief that tannin is present. And it is probable that this behavior and Prescott's report became the basis of statement that cascara sagrada contains "a tannic acid."

Hooper's opinion (*Pharm. Jour. & Trans.*, 3rd ser., vol. 18, p. 681 (1888)) that the bark of *Rhamnus Wightii* contains more tannin than that of *R. Purshiana* does not appear to have been formed from an actual comparison of the two barks, but was drawn by interpreting the presence of "tannic substances" from the reactions obtained with iron salts when making an examination of the bark of *R. Wightii* alone.

Illustrative of the possible uncertainty in the recognition of cascara sagrada, as late as 1892, Maisch ("Organic Materia Medica"), in describing the barks of *R. Purshiana* and *R. californica*, said that "both these barks when masticated color the saliva yellow and resemble also frangula bark in their behavior to alkali and ferric chloride." And, following this remark, "tannin" is given prominence as the first-named in his list of constituents of cascara sagrada.

When enumerating the constituents of the fruit of *Rhamnus catharticus*, commonly called buckthorn berries, Maisch (*loc. cit.*) names among others "rhamnin (yellowish granules, tasteless, olive-green by ferric salts)" and he also mentions "tannin," but makes no comment on the deportment of it toward ferric salts or other reagents.

It is very evident, however, that pharmacognosists soon learned that the assumption of the presence of tannin from the behavior with ferric salts is not altogether warranted, for not one of the several who have dealt with cascara sagrada so ably and exhaustively has made any mention of the use of ferric compounds as a means to recognition or of differentiation. This avoidance becomes an impressive fact when the intensity of the reaction as shown by whole or ground bark, or by the decoction, is considered.

As regards pharmacognostical reference to tannin as a constituent of cascara sagrada, Perrot (*Jour. Pharm. Chim.* (6) 13, 161), as abstracted in PROC. AMER. PHARM. ASSOC., 1901, p. 741, is quoted as follows—"The powder of the latter bark (*R. frangula*) contains numerous collenchymatous cells and corky layers markedly impregnated with red-brown tannin, which are not present in the bark of *Rhamnus Purshiana*."

But reference to the original article reveals the fact that Perrot reported the presence in the bark of *R. frangula* of a "rouge-brun" "matiere tannoide," and in the bark of *R. Purshiana* of a "jeune-brun" "matiere tannoide;" but he makes no statement on the means whereby he reached the conclusion that the cell contents of either drug is "matiere tannoide," while this qualified designation of the two substances implies "tannin-like" rather than "tannin."

Another mention of tannin as a constituent of this drug was made by Dohme and Engelhardt in their contribution to "The Chemistry of Cascara Sagrada," which they presented to the AMERICAN PHARMACEUTICAL ASSOCIATION in 1897 and published in the ASSOCIATION'S PROCEEDINGS for that year. Their reference to tannin will be quoted when a description of our repetition of this part of their work is given.

In 1904, Jowett presented to the same ASSOCIATION his "Chemical Examination of Cascara Bark." In this study, which was published in the A. PH. A. PROCEEDINGS for that year, Jowett's only mention of tannin as a constituent was in noting 2.4 and 3 per cent of "tannin" for the two samples of this bark (aged 1 and 3 years, respectively), which he determined by the hide-powder absorption method (while a 2-year old sample of bark of *R. californica* gave indications of 3.9 per cent).

He used precipitation by lead acetate and subacetate as means to the observation of those fractions of his material in which he would have expected to encounter any tannin that the drug might contain; and he recovered by action of hydrogen sulphide from these precipitates certain substances which he described as "regenerated;" but though he made no reference whatsoever to tannin as present

in any of the material so examined, he stated specifically that emodin was "the only definite product isolated directly from the lead acetate precipitate."

Jowett also reported that the active principle or principles of cascara "are contained in that portion of the alcoholic extract which is soluble in water and precipitated by lead subacetate" and that "furthermore, it is contained in that portion of the regenerated lead subacetate precipitate which is soluble in ethyl acetate." It is well to note that in this fraction would likely have been any water-soluble tannin that had escaped precipitation by lead acetate. And it is particularly noteworthy that his water-soluble portions of the ethyl acetate extract of the substances regenerated from both the lead acetate and lead subacetate precipitates gave dark-brown colors with ferric chloride.

Immediately following the reading of Jowett's results to the ASSOCIATION, Nelson presented to the same meeting his "Notes on The Pharmacology of Cascara Sagrada and Bitterless Preparations of Cascara" (PROC. AMER. PHARM. ASSOC., 1904).

Nelson extracted the drug with warm "absolute" alcohol, then with 75% and thereafter with diluted alcohol; from the mixed extracts the alcohol was recovered, and the residue well shaken with 20% alcohol and powdered pumice. The filtrate was precipitated with lead acetate, this precipitate washed and mixed with solution of sodium carbonate, the mixture filtered, the filtrate neutralized with acetic acid and evaporated on sand.

The fraction of the drug so obtained was regarded by Nelson as containing tannin; not, however, from evidence by any test that he applied, but partly by the inference that at this point "tannins, plant acids, glucosides, colors, etc." might be found, and mainly by the assumption that the purgative effect of this fraction "might have been enhanced by removing the tannins."

In making this statement, Nelson may have accepted the opinion of previous investigators, since he refers especially to the work of Dohme and Engelhardt, who seven years before had reported what they believed to be "a mixture of the lead salt of the glucoside with the lead salts of the tannates present."

The foregoing notes summarize the evidence which the literature of cascara sagrada contains to indicate by either direct statement or implication that tannin is a constituent of this drug. There is no record of any special attempt to isolate and purify any such substance, nor of any undertaking to examine the indication of its presence which ferric compounds give, and upon which behavior alone the affirmation of the presence of tannin seems to have been made.

It is a remarkably consistent observation by all who have sought the active principle, that this is fractioned into that part of the extractive which would also contain any tannin of conventional properties; but equally remarkable is the scant attention which this fact has received.

The bark which was used in the work about to be reported complied with official requirements including that of age; it had been collected nearly four years prior to this use of it.

When regarding a drug for tannin, a study of its decoction is always of interest, for this preparation offers opportunity to gather some of the early impressions with respect to the presence and amount of this constituent.

Decoctions of cascara sagrada have odor suggestive of the drug and very

faithfully represent its bitterness. The decoctions give blackish brown colors and brown precipitates with ferric chloride test solution, and almost identical behaviors with ferric acetate solutions.

Gelatin solution precipitates the decoctions; the stronger decoctions at once, and the weaker within a few minutes.

All decoctions of cascara sagrada are rendered cloudy by acidification with acetic, hydrochloric, oxalic or sulphuric acid; and if the decoction be first acidified with acetic acid and the mixture be allowed to stand until the precipitate has settled, the filtered liquid will give much less precipitate with gelatin and it will be of a much lighter color. In this acidified and filtered liquid, ferric chloride produces the characteristic blackish or dark brown color, without causing a precipitate.

The precipitation of extractive from the decoction by simple acidification is even more pronounced when hydrochloric acid is used and while ferric chloride continues to cause the blackish and dark brown colors, gelatin will not always give a precipitate.

The ten per cent decoction, allowed to cool and filtered clear, is brilliant and has a dark wine-red color. It is distinctly acid to litmus paper, and slowly produces effervescence with calcium carbonate. To circumvent any interference by free oxalic acid or acid oxalates of the ferric chloride test for tannin, some of the decoction was treated with an excess of calcium carbonate, and after several hours of contact was filtered and refiltered to obtain an entirely clear liquid. This specially prepared fluid gave with ferric chloride the same color and precipitate as were shown by the original decoction; it also precipitated with gelatin, and was still precipitable by acetic acid.

The effect of hide-powder on the ten per cent decoction was striking. Immediately, the greater part of the color was absorbed, causing the dark wine-red color to become of a brownish amber or diluted caramel color. After two hours contact, it was noted that approximately four-fifths of the color had been taken out of solution. There may be a selective withdrawal of the color, as simple dilution of the original decoction failed to match exactly the tint of the partly decolorized fluid. Some of the acidity of the decoction was absorbed by hide-powder, but there was little difference detectable in the element of bitterness.

In the hide-treated liquid there was less precipitate by ferric chloride, but still a production of the characteristic color, the blackish shade of which was now found to be more likely noticeable by reflected light; as transmitted light showed but dark-brown colors, without the peculiar feature of blackness.

The iron color-reacting factor is thereby shown to be non-absorbable by hide-powder. The clarity of the hide-treated liquid was not disturbed by acetic, hydrochloric or sulphuric acid. The mixture with the last named acid actively decolorized potassium permanganate.

Gelatin produced no precipitate in the hide-treated liquid, a behavior which shows that the iron color-reacting factor and the gelatin-precipitating property do not reside in one and the same constituent.

The Procter hide-powder method of estimating the "tanning value" of vegetable substances was applied to a ten per cent decoction with the resulting absorption of 2.28% of the bark represented. But, as is well known, these bare

figures mean nothing positive as to the presence of that amount of actual tannin, because this quantitative process is subject to variations which are greater than this amount, on account of irregular absorption of extractive matter by the hide.

An approximately saturated infusion of cascara sagrada prepared with cold water and carefully filtered is a brilliant liquid with deep wine-red color, pronounced acidity to litmus paper and intense bitterness. This clear liquid becomes cloudy upon simple dilution with water, either at once or very soon; it also clouds upon acidification with acetic acid, also by addition of gelatin, and even by mere warming.

As the process of dialysis also offers opportunity to observe the behavior of tannins and associated substances, some of the ten per cent decoction was placed on a parchment septum arranged so that the liquid within and the water without the membrane would maintain the same level.

Immediately an osmosis of color began, and from this first appearance of color in the outer liquid, the latter showed the characteristic color reaction with ferric chloride; but in this dilution gave no precipitate with gelatin. When, however, this slightly colored fluid, or a subsequently obtained one, was evaporated to a proportionately small bulk, as for instance to one-tenth of its volume, the color of the resulting liquid was much intensified, and the cold and filtered fluid not only gave a darker color and heavier precipitate with ferric chloride, but, also in the case of the first several lots of it, gave precipitates with gelatin. When finally gelatin ceased to give these precipitates, ferric chloride continued to produce the peculiar dark-brown color.

From this experiment, and, from the additional observation that the coloring which is not absorbed by hide is augmented by evaporation, it would seem that some part of the coloring is a result of change in, perhaps an oxidation of, a dialysable constituent originally present, or produced when the bark is brought into contact with water. Such may be a result, if not the usual understanding, of the changes sought through the aging of the bark.

None of the highly colored fluids obtained by evaporating the slightly colored watery dialysates could be rediluted with water or acidified with acetic acid without precipitation taking place. All of these concentrated solutions gave with potassium hydroxide solution the red color indicative of emodin.

After four days on the septum, with three changes of water daily in the outer vessel, the remaining liquid was filtered, as it now showed suspended matter. The filtered liquid was fluorescent, had an opalescent turbidity, was light brownish-yellow, and had an acid reaction to litmus paper and a bitter taste. It did not precipitate gelatin, but gave copious, flocculent or almost gelatinous precipitates with ferric chloride solution and with acids; but there was only the faintest indication of the color usually produced by ferric chloride, thus attesting the complete passage or change of the factor which causes the color reaction with ferric compounds. As this residual liquid gave a dark red color with potassium hydroxide solution, it very likely contained some emodin.

After these preliminary observations, several means for extracting and isolating tannin were tried. Ground drug (2.5 kilo.) was boiled for half an hour with each of three successive portions of water of double the weight of drug; the strained liquids mixed, evaporated to about the weight of the original drug, this solution

strained and allowed to become cold. When cold, this solution was again strained, then shaken with several successive portions of acetic ether (the ethyl acetate of the references made herein).

Upon separating this solvent and evaporating it under reduced pressure, it yielded a reddish residue, almost varnish-like, containing an abundance of minute crystals arranged in burr-like formation. While there was no manifestation of the presence of tannin by any appearance of this residue, due allowance was made for the presence of crystalline matter, which, as experience has taught, may militate against if not completely prevent the "puffing" of residues containing tannin when the solvent is removed *in vacuo*.

Ether applied to the residue removed at once the greater part of the red color, but a less considerable portion of the substance itself, giving a brilliant red solution. Subsequently applied portions of ether continued to remove yellow color and substance, and this treatment was repeated until the lemon color of the ethereal solution was apparently constant, for it failed to produce a sharp separation, although the substance producing the continuous color was but of trifling amount in the last washings.

When the red ethereal solution was evaporated, there was left a residue of reddish crystalline and granular substance, which imparted a yellowish or amber tinge to water; the solution so obtained gave the characteristic dark olive-brown color and some precipitate with ferric chloride, and a slight precipitate with gelatin solution upon standing for an hour.

The lemon-colored ethereal solution upon evaporation gave a similar residue, but with less red color, which in water solution had almost the same behavior toward ferric chloride, but did not show precipitation with gelatin even upon allowing the test to stand for an hour.

These crystalline and granular substances dissolved in weak solutions of potassium hydroxide with production of purplish-red or blood-red solutions, which gave flocculent orange-colored or yellowish precipitates when acidified.

That portion of the water extract which was removed by acetic ether and thereafter left undissolved by the several lots of ether, gave a porous residuum when dissolved in acetic ether and distilled *in vacuo* to remove this solvent. But neither the initial appearance of this residue on the wall of the flask as the solvent gradually evaporated nor the behavior of the last few drops as the solvent was finally dissipated conformed to the characteristic behaviors of tannins at these stages.

This residue was but sparingly soluble in water to which it imparted a yellowish color and a bitter taste. As further attempt at purification or separation, it was repeatedly treated with ether which removed small amounts of yellow matter, and with chloroform and benzol, both of which also dissolved from it some similar substance.

Between these treatments, the residue was rubbed with water and acetic ether, into which latter solvent it went with readiness and with but little diminution through any effect of the water. Upon separating and evaporating the acetic ether layer under reduced pressure, the porous resin-like substance was easily reobtained.

Several repetitions of these treatments made but little if any difference in either the appearance or behavior of the substance, but water triturated with the

substance differed from the decoction in that the water-soluble part did not cloud with gelatin solution (even upon standing and acidification with acetic acid) although it continued to give the characteristic color reaction with ferric chloride.

As the final product, the substance was obtained from acetic ether solution in the form of a light brown, porous mass which filled the flask through its spongy-appearing but really very fragile state. It was obtained in more of a puffed and glistening tannin-like appearance by moistening it with ether and rapidly evaporating the solvent *in vacuo*. (A specimen of each state was shown.)

To further examine the original decoction from which this substance had been obtained, by shaking with acetic ether, the aqueous layer from which the latter solvent was drawn off was now strongly acidified with acetic acid and agitated with additional lots of acetic ether. There was, however, but little more substance removed, and after the above-mentioned treatment it had the same properties as the porous resin-like product which has just been described.

Next the aqueous liquid remaining from the original decoction was mixed with another portion of acetic ether and thereafter saturated with sodium chloride; and again only a trace of material was removed, and after the described treatment resulted in the same product.

Another lot of the ground bark, 2 kilo., was percolated with acetic ether until exhausted of bitterness, by which time color and weighable extractive had been removed.

The solvent was recovered and the tarry-black mass treated with chloroform, which took away greasy material that was found to be almost insoluble in water, although this solvent carried into solution some of the substance giving the characteristic color reaction with ferric chloride; but the solution thus reacting did not show any tendency to precipitate with gelatin.

The chloroform-insoluble part of this acetic ether extract was examined in two ways. One half of it was treated with ether which dissolved much of the dark-colored substances, and, after repeated treatments with water and acetic ether, left a resin-like residue identical with that obtained by shaking the decoction with acetic ether and purifying as first described.

The other half was treated with water, the mixture filtered to remove the undissolved matter which appeared to be almost the entire substance. The clear golden-yellow filtrate was shaken with acetic ether. This solvent removed but a trifling amount of a yellow, viscid substance, which, judged by a control experiment made by evaporating some of the liquid, was the only matter in the water solution.

The golden-yellow filtrate gave the characteristic color reaction with ferric chloride, but no cloudiness with gelatin.

By treating with ether and thereafter with acetic ether in the presence of water, the resinous substance obtained from this half of the acetic ether extract of the drug resulted in a product like that first had from the decoction.

The amount of this final product of the acetic ether extraction of the drug was relatively much larger than that from the decoction.

Having noted that acetic acid caused precipitation in decoctions of cascara sagrada, a third portion of the ground drug, 1 kilo., was macerated with 6 per cent acetic acid for two days. A clear, dark wine-red liquid was then filtered off;

it reacted characteristically to ferric chloride and gave a precipitate with gelatin solution.

This acetic acid solution of the bark was shaken with acetic ether which removed but a small amount of substance, that, carried through the method of purification already described, yielded a trifling amount of the resinous product obtained from the other processes of extraction.

There was no evidence of phlobaphene nor of tendency toward formation of such substance anywhere in the foregoing attempts to isolate and purify any tannin constituent of the bark; but it was decided to learn what might be done toward obtaining this form of tannin from *cascara sagrada* by extracting with water in the presence of alkali.

Accordingly, an experiment was made by mixing ground bark with magnesium oxide and extracting with hot water after the manner prescribed for the preparation of aromatic fluidextract of *cascara sagrada*.

The alkaline extract so obtained from 1 kilo. of the drug was concentrated by evaporation to 1 litre, then nearly neutralized with hydrochloric acid and finally strongly acidified with acetic acid. The resulting turbid liquid was shaken with acetic ether, which removed a substance similar to that from the other methods of extraction, but in very small proportion.

In the course of this work, it was found that most water solutions of *cascara sagrada* that contain enough extractive to impart wine-red or reddish-amber colors give at once precipitates with gelatin solution; while those solutions with but amber or yellowish colors, that is without reddish tinge, if freed from turbidity, as by repeated filtration, give no precipitate, unless, on prolonged standing.

But this peculiarity of gelatin to combine or precipitate with extractive is by no means a recent observation, for such behavior was noted on the part of tanning materials, a century ago, by Sir Humphrey Davy and his contemporaries, who were seeking to put leather-making on a scientific basis.

The irregular behavior of gelatin that was observed in this work may possibly have been due to the presence of emodin in the water solutions along with other extractive matter from the drug through mutual interaction as solvents for each other; for, as pointed out by Jowett, "emodin, although insoluble in water, is soluble in the aqueous extract of the bark, and that it is extracted from such a solution only slowly and with difficulty by shaking with immiscible solvents such as benzene, ether or chloroform;" and he referred to this peculiar behavior of emodin as "a possible explanation of the results of previous observers."

Having so far failed to obtain a tannin by any of the foregoing methods of extraction, and having recognized the reason for the irregularity of the gelatin test, the reference to the presence of "tannin" by Dohme and Engelhardt was next considered. While their mention of "tannin" was but casual in connection with their pursuit of the active principle, it is, nevertheless, next to the statement of Prescott, the most specific of those in the literature of the drug.

They proceeded as follows as quoted from their report: "The drug was extracted with chloroform to remove fats, etc., and the residue extracted with 80 per cent alcohol and the resulting extract dried and dissolved in hot water. On cooling, some resinous, waxy substance separated and was filtered off. The filtrate was treated with lead acetate, which produced a yellow precipitate. This

was filtered off and stirred with hot water on a water-bath. As the lead tannates are difficultly decomposable by H_2S it is advisable to pass this gas through the suspended precipitate of lead salts at a temperature of about $100^\circ C$. This was done until on shaking the flask, whose mouth was closed by the thumb, the latter was raised by the pressure of the gas. The PbS was filtered off and the filtrate evaporated to dryness, resulting in a dark brown substance which consisted mainly of tannins, as portions of them dissolved in water gave good inks on treatment with ferric salts. This tannate mass appears to be composed of several tannins which we did not undertake to investigate."

Operating on 900 grams of the finely ground drug, their procedure was repeated in every detail, except that of closing the flask with the thumb, instead of which the contents of the flask were constantly stirred with the glass tube through which the H_2S was passing, and this treatment was continued on a boiling water-bath until long after complete decomposition of the lead-cascara precipitate had been shown by every indication of change and final constancy in appearance, as well as by a superabundance of hydrogen sulphide.

The chloroformic extract was a dark greenish brown semi-solid mass. Water applied to it acquired only a slight greenish yellow tint. This solution was acid to litmus, gave no precipitate with gelatin nor lead acetate, and only a faint greenish color with ferric chloride.

The 80 per cent alcohol removed all bitterness from the drug, but not all of the iron and gelatin-reacting substances as will hereinafter be mentioned in detail. This alcoholic extract amounted to about one-fourth of the weight of the drug.

The turbid mixture of the water-soluble part of the alcoholic extract with the comparatively small amount of undissolved matter was treated with a slight excess of lead acetate solution, which made an olive-green precipitate. Upon filtration, the fluid part was obtained clear, where before the addition of the lead salt the turbidity was not easily removable by filtration. The clear filtrate had a dark wine-red color. The lead precipitate was washed by suspension in two successive portions of water, which upon decantation were added to the original filtrate to obviate any loss of the principle being sought. The lead precipitate was then decomposed with H_2S , as explained, the lead sulphide filtered off, and the wine-red liquid evaporated to a syrupy consistence, which on cooling became a semi-solid mass with the characteristic color of cascara extract. This residue was but partly soluble in acetic ether, which removed from it a resin-like substance similar to that obtained as the end-product of the several methods previously tried.

Rubbed with acetic ether and water, most of the residue which was not dissolved by acetic ether alone passed into solution; the much smaller undissolved portion was soluble in alcohol. When the acetic ether layer was separated and this solvent recovered, the familiar resin-like substance was again had.

The dark wine-red filtrate from the precipitate caused by lead acetate in the water solution of the 80 per cent alcohol extract was treated with lead subacetate solution which gave a salmon-colored to carrot-colored precipitate which settled out much more quickly than did the precipitate caused by lead acetate. The precipitates by both the lead acetate and subacetate were soluble in acetic acid. The filtrate from the lead subacetate precipitate had a reddish amber

color and gave additional precipitate when partly neutralized with potassium hydroxide. And even after removal of this last-named precipitate, the filtrate gave the characteristic reaction with ferric chloride.

The precipitate made by lead subacetate was more easily washed, and when suspended and decomposed by H_2S gave a brilliant dark-amber solution.

Upon evaporation of the filtrates from the lead sulphide precipitates, there was found to be little apparent difference in the solid extracts thus obtained from the lead acetate and subacetate precipitations; although a much larger proportion of that from the use of the subacetate redissolved upon addition of water.

The water solutions representing both lead precipitates, both in the original unevaporated and in the solutions obtained by redissolving the solid extracts, gave the characteristic reaction with ferric chloride and precipitated gelatin solution; but while acetic ether removed from these solutions the substance which gave the reaction with ferric chloride, it did not carry with this behavior the ability to precipitate gelatin.

The reaction obtained with ferric chloride was, in the case of the substances from both lead precipitates, the production of a brownish precipitate in dark olive-brown liquids; and although there was in some instances a suggestion of blackness when the test was viewed by reflection, still there was never observed a single reaction that showed by transmission the black, blue or green shades usually associated with reactions for tannin. While we have worked with tannins which were difficult to purify, and which continued to give variations in browns from the mentioned usually accepted colors (*Am. J. Pharm.*, p. 313 (1896)), we have always found these substances to give precipitates with gelatin.

It will be seen by the foregoing notes that we have not been able to confirm the presence of a tannin by following the procedure of Dohme and Engelhardt. And it is noteworthy that Jowett also secured "dark-brown" colors with ferric chloride from the materials which he regenerated from the lead acetate and lead subacetate precipitates obtained from comparable material.

The drug which had been exhausted with chloroform and 80 per cent alcohol, when freed from this solvent and boiled with water yielded a dark wine-red solution that gave the characteristic color with ferric chloride and some turbidity with gelatin, but here again extraction by acetic ether and purification by repeated solution in it brought about a separation of the two reactions, and resulted in a small yield of the resin-like substance which has been uniformly the ultimate product of our attempts to extract a tannin from cascara sagrada.

The bark used by Dohme and Engelhardt was described by them as "a typical specimen." It was less than one year old. But Jowett stated, as the result of his work, that "no difference could be observed between the chemical characteristics of the fresh (one year old) or of the so-called matured bark (3 years old)."

This final product, evidently a mixture, and referred to as resinous or resin-like merely as means of mention and without thought of classing it, seems to be the factor that at every point gives what has been described as the characteristic reaction with iron salts, which effect has been interpreted by some investigators as evidence of tannin.

This substance was soluble in alcohol, with production of an amber or yellow

solution, which withstood dilution with water in all proportions. The alcoholic and dilute alcoholic solutions gave the peculiar color reaction and precipitate with ferric chloride and a deep flesh-colored precipitate with lead acetate. Acetic acid dissolved the substance to make a yellow solution which was immediately precipitated upon dilution with water. Acetone and acetic ether dissolved it with deep wine-red colors. It was largely soluble in glycerin, but this solution was precipitated by addition of water.

From the yellow-tinged water solution of this substance, hide-powder did not absorb either this color or the property of reacting characteristically with ferric chloride.

Furthermore, when subjected to dialysis the saturated water solution of this final substance gave a dialysate similar to that from the decoction, in so far as the reaction with ferric chloride is concerned, but differed in behavior toward gelatin, for the evaporated liquid, although it developed an intensified color through concentration, did not show precipitation with gelatin; again showing that the iron color-reacting and gelatin precipitating effects do not reside in the same substance.

The outcome of this work has been to find that the substance or mixture which gives the reaction with ferric compounds is (1) non-absorbable by hide powder, (2) that it is dialysable through parchment and (3) that its saturated water-solution does not precipitate gelatin.

In view of these attributes, so distinctly negative for tannins, and of the explanation for the irregular behavior of the gelatin test, it seems conclusive that *casacara sagrada* does not contain a tannin.

A CONVENIENT APPARATUS FOR BLOOD COUNTING.

BY E. R. SERLES.

Everyone who has been in a clinical laboratory has at some time or other been called upon to make a blood count. Under the old method of filling the pipettes by means of the rubber tube and human lung power, many difficulties are encountered. The novice finds it very difficult to watch the tiny column of blood rise to 05 and at the same time hold the rubber tube so that there are no kinks which will cause him trouble when he places his tongue on the opening in the mouth piece.

In order to eliminate the difficulty mentioned above, the author substituted for the lung power a 2-cc. hypodermic syringe and shortened the rubber tube to about one inch. This worked much better, but the flexibility of the rubber tube still caused slight changes in the length of the air column above the blood and gave rise to errors when the pipette was carried from the blood droplet to the diluting fluid.

The new apparatus, which consists of an ordinary haemocytometer pipette with the top end reinforced and ground to fit the nozzle end of a 2-cc. Luer syringe, makes an ideal piece of equipment.

In order to insure perfect connection, the nozzle of the syringe barrel A may be coated with a film of castor oil. This not only insures a close fit of the two parts, but it allows for the easy removal of the syringe when you wish to transfer it to other