AN ABSTRACT OF THE THESIS OF

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Ephedra Viridis Coville Found in Oregon

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A species of Ephedra found in southeastern Oregon, identified as Ephedra viridis Coville is reported of medicinal value among the American Indian tribes and other dwellers of that locality in various affections such as venereal diseases, kidney and bladder disturbances, in stomach ulcers and abdominal pains, in delayed menstruation, as blood tonic, as an aid in the relief of rheumatic pains; also in cases of diarrhea in children and for colds.

A preliminary phytochemical investigation of this plant, Ephedra viridis Coville, has been undertaken in this report, the purpose of which is to establish the characteristics of the plant and determine its alkaloidal content (ephedrine).

A general plant analysis of the drug samples yielded the following results: Moisture, 5.5 to 7 per cent; total ash, 6.5 to 7.9 per cent; water-soluble ash, 0.86 per cent; alkalinity of water-soluble ash, 0.932 (mls. of 0.1 N HCl per gram sample of the drug); water-insoluble ash, 7.12 per cent; alkalinity of water-insoluble ash, 12.323 (mls. 0.1 N HCl per gram sample of the drug); acid-insoluble ash varied from 0.075 to 0.24 per cent, volatile oil constituents varied from 0.29 to 2.4 per cent, the crude fiber content of the drug, 17.3 per cent. The determinations of total extractives with different solvents gave the following results: alcohol-soluble extractive, 33.2 per cent, dilute-alcohol extractive, 17.4 to 23.53 per cent, total ether extractive, 2.765 per cent, volatile-ether soluble extractive portion, 0.32 to 0.34 per cent, non-volatile-ether soluble extractive, 2.5 per cent, petroleum ether extractive, 1.7 to 2.2 per cent, and water-soluble extractive, 16.3 per cent.

The alkaloidal content of this sample of Ephedra viridis Coville, determined according to the official assay of bella donna leaf varied from 0.02 to 0.299 per cent. Because of this insignificant alkaloidal content, the therapeutic value (with regards to ephedrine) of this plant by the Indian tribes appears questionable.

A PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF EPHEDRA VIRIDIS COVILLE FOUND IN OREGON

by

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A PRELIMINARY PHYTOCHEMICAL INVESTIGATION OF EPHEDRA VIRIDIS COVILLE FOUND IN OREGON

INTRODUCTION

Ephedra is the whole plant of a single genus of the family Ephedraceae inhabiting the arid regions of the northern hemisphere (1, p. 77).

Seventeen species are known in the Old World, six in North America (in the arid and semi-arid regions) and eight in South America (56, p. 134). In the United States, the known native species of Ephedra are E. antisyphilitica Meyer, (in Arizona, New Mexico and southern California), E. Californica Watson (California and Colorado), E. viridis Coville (California, Nevada, Oregon, Arizona, Utah, Colorado), E. nevadensis Watson (California, Nevada, New Mexico, Utah, Arizona, Colorado, Oklahoma and South Dakota); other species native in these regions are E. pedunculata Engelm, E. torreyana Watson, E. trifurca, Torrey (16, 31, 46, 55).

In China, the Ephedra plant is commonly known as "Ma Huang" (meaning yellow astringent) (21, p. 117) and has been used in medicine since ancient times. According to K. K. Chen, (51, p. 182), it was tasted by Emperor Shen Nung over 5000 years ago.

As a follow up of the work done by Yamanashi (50, p. 718), Nagai, in 1887, obtained an alkaloid from Ephedra (Ma Huang) which he named "Ephedrine". The value of this active principle in the treatment of asthma and other affections, and its action simulating that of epinephrine, even with certain advantages over the latter, (11, 32, 51, 60, 55), has encouraged the continued investigations of different species of Ephedra found in different regions, for the development and cultivation of possible commercial source of the alkaloid ephedrine.

The chief sources of Ephedrine (27, pp. 643-645; 60, p. 425) are Ephedra sinica Stapf (Ephedra vulgaris var. helvetica or E. vulgaris), E. equisetina Bunge (also known as E. monosperma Gmel. and E. monostachya L., (52, pp. 163 - 167), E. intermedia Schenk (and Meyer, or E. pachyclada) (10, pp. 647 - 651) and other species (Old World). The commercial sources of the alkaloid ephedrine are known to be native species growing in China (23, pp. 233 - 248; 51, pp. 182 - 187; 52, pp. 163 - 167; 53, pp. 27 - 30), India (9, pp. 889 - 894; 10, pp. 647 - 651; 20, pp. 1189 - 1192; 22, pp. 636 - 641), and Syria. Different species native in Europe - Italy, Sicily, and Sardinia (6, pp. 472 - 473; 7, pp. 431 - 433; 15, pp. 684 - 688; 25, p. 294; 37, pp. 66 - 71; 42, pp. 315 - 334; 51, pp. 182 - 187; 54, pp. 135 - 137; 59, pp. 327 - 328), are known also to contain appreciable amounts of the alkaloid ephedrine besides its

isomers. Likewise, the species found in northern Africa and South America have been reported to contain the alkaloid and its isomers (45, pp. 19-28; 3, pp. 129 - 142). Experiments in the cultivation of Asiatic species of Ephedra are being conducted in the United States for the possible commercial source of Ephedrine (4, pp. 171 - 173; 13, pp. 199 - 209; 26, p. 16). Several native species of Ephedra have also been found in the United States reported of medicinal value. The American Indian tribes used certain of these plants in the treatment of certain diseases (55, pp. 62 - 129). For instance, Ephedra nevadensis Watson and Ephedra viridis Coville and other species, are brewed as tea and taken as treatment of venereal diseases, used in kidney disturbances and bladder disorders as a diuretic; also for colds, as blood tonic, as a remedy in stomach disorders and ulcers, in delayed menstruation; as an aid in the relief of rheumatic pains, and in cases of diarrhea in children. However, earlier investigations have reported that most of these native species contained none (4, pp. 171 - 173; 46, pp. 819 - 821) or negligible or only traces of the alkaloid.

Because of the medicinal values mentioned above and the importance, in particular, of the alkaloid Ephedrine in modern medicine, interest has been shown in the determination of the Ephedrine content of different species of

Ephedra. In this particular report, a preliminary phytochemical investigation of Ephedra viridis Coville, found in southeastern Oregon, seeks to determine the presence of the alkaloid and to establish the characteristics of the plant (this species).

EXPERIMENTAL

Ephedra viridis Coville is a xerophytic dioecious shrub (32, p. 60), growing in the desert and in the Juniper belt of the Mojave Desert region, Upper Sonoran zone, Southern Nevada to the vicinity of Fort Tejon and the desert slopes of the San Bernardino Mountains, California (1, p. 77); Arizona and Utah (31, p. 61), in desert ranges, mostly 5000 to 7000 feet (32, p. 60; 16, p. 220). It was first seen on a sheltered north slope of a peak near Copper City Spring and afterward in the Funeral Mountains on a peak west of Amargosa, in Charleston, Panamint, Inyo and Coso Mountains, on the eastern slope of the Sierra Nevada near Lone Pine, in Walker Pass on the divide between Kernville and Havilah. Dr. Merriam found it also in the White Mountains of California, Gold Mountains and Mt. Magruder, Pahranagot Mountains, and the Highland Range of Nevada, and the Beaverdam Mountains of Utah (16, p. 220).

Two years ago, Mr. G. A. Hagey, member, Board of Pharmacy Inspectors, Portland, Oregon, brought samples of

this species of Ephedra to the School of Pharmacy, Oregon State College. Its identity as Ephedra viridis Coville was verified and certified by Dr. Albert Steward, curator, Herbarium, Botany Dept., Oregon State College. The plant material under investigation was obtained in southeastern Oregon (Pueblo Mountains). It is reported that the dwellers in that region and in the vicinity have used it in the treatment of certain diseases as has already been mentioned previously.

The plant samples under investigation, (without flowers or fruits), were compared with the collection in the Herbarium, Botany Dept., Oregon State College, and meets the following description (1, p. 77; 16, p. 219; 31, p. 61; 32, p. 60): Ephedra viridis Coville is a dioecious erect shrub, 0.5 to 1 m. high, with numerous slender, jointed, erect, broom-like scabrous bright green branches. The leaf scales are opposite, 3 to 6 mm. long, connate or sheathing for apparently 2/3 their length when young, usually broken in later age but the brown thickened base is persistent (obtuse towards the end of branches tapering at the apex). The staminate aments (male flowers) or catkins are sessile with 4 or 5 pairs of yellow bracts. The fruiting bracts (female flowers) are 4 pairs, round, ovate, sessile and green. The fruit (not available) is often in pairs and triangular, that is, one or two in a

catkin with flat faces and carinate back (convex), 3 1/2 to 4 lines long.

A Preliminary Chemical Investigation of Ephedra Viridis Coville.

A. Methods and Proceedures

1. Moisture determination. Concordant results of the determination of the moisture content of the drug were obtained, using the "Toluene distillation method" (57, pp. 777 - 779; 30, pp. 358 - 371), and the dessicator drying method (8, pp. 189 - 194). By toluene distillation, approximately 7 per cent water content for both samples of the June and October collections was obtained. The samples of the October collection, however, yielded a lower percentage of water, 5.5 per cent, by the dessicator method than those of the June collection which yielded about 7 per cent. (For the October collection, weights of the dessicator dried drug became constant within 45 days, while that of the June collection became constant within 50 days.) (See Table 1, a, b)

2. Ash determination (30, pp. 351 - 358).

a). Total ash. This is the residue left after complete incineration of the plant material. An average of approximately 6.3036 per cent and 7.8741 per cent of the total ash of the June and October collected samples, respectively, were obtained. (See Table 2, a)

b) Water-soluble ash and its alkalinity. The difference in weight between the total ash and the water-insoluble ash, (after water treatment) (61, pp. 23 - 27), represents the water-soluble ash contents of the drug. Its alkalinity, determined by titrating the aqueous filtrate with 0.1 N HCl, using methyl orange as indicator, is expressed in terms of mls. of 0.1 N HCl required to neutralize 1 Gm. sample of the drug. An average of 0.9318 mls. of 0.1 N HCl per gram sample of the drug was required to neutralize the water-soluble ash contents. (See Table 2, b)

c) Water-insoluble ash and its alkalinity. The dried residue after water treatment and separating the aqueous solution of the total ash, represents the water-insoluble ash. The number of mls. of 0.1 N HCl taken up by the water-insoluble ash (calculated from the result of the residual titration with 0.1 N NaOH) represents its alkalinity and is expressed as the number of mls. of acid to neutralize the insoluble ash per gram sample. (See Table 2, c)

d) Acid insoluble ash. The undissolved, dried residue, after HCl treatment of the water-insoluble ash, constitutes the acid-insoluble ash. (See Table 3, c)

The sample of drug submitted to the ash determination was the residue left after the moisture determination

by dessication and which was later heated on the water bath to volatilize the volatile oil contents (volatile oil determination, 8, pp. 189 - 194).

3. Volatile Oil Determination (8, pp. 189 - 194; 57, pp. 780 - 781).

Two methods were employed, steam distillation, (57, pp. 780 - 781), and steam-bath volatilization (8, pp. 189 - 194). The first method, mentioned, yielded only traces which could not be read possibly (only a small quantity of the drug was used as available) since it formed a turbid mixture with the water that distilled over with it, even upon attempts of salting it out. The steam-bath volatilization of the dessicated samples yielded a loss in weight ranging from 0.3 per cent to 2 per cent (there were variations), representing the volatile oil contents of the drug. (See Table 3)

4. Crude fiber content (30, pp. 378 - 383; 57, p. 779).

The dried weighed residue left undissolved after successive thirty minute treatments with boiling acid (1.25 per cent H_2SO_4) and alkali (1.25 per cent NaOH) was incinerated to constant weight at about 500°C., the loss in weight during this treatment then being calculated as crude fiber content of the drug, which was approximately 17.3 per cent.

Formula:

$$\frac{\text{Residue minus ash}}{\text{Weight of Sample}} \times 100 = \text{per cent crude fiber}$$

5. Extractives. (30, pp. 372 - 378; 57, pp. 779 - 780.

a) Alcohol (95 per cent). In general, the resinous constituents of plants is extracted by means of alcohol. Volatile acid constituents (30, p. 375), react with the alkali introduced into the receiving flask of the "continuous extraction" set up, forming the corresponding salts.

The alcohol extractive was at first dark green but turned reddish brown and turbid upon interaction with the alkali in the flask. The residue in the thimble was almost colorless (fibrous) but also brown-red speckled marc. The residual alcohol extractive, when evaporated to dryness (spontaneous) was chocolate red in color, giving a clear wine red aqueous solution. The residual alcohol solvent decanted and filtered from the alkali-alcohol residual extractive was evaporated to dryness, yielding a yellowish and brownish residue. (The thimble with the insoluble residue absorbed moisture very rapidly especially after drying it in the oven.) An average of 33.2 per cent alcohol extractive was obtained. (See Table 5)

Formula:

a) Subtract: original weight of drug

Minus

moisture content of the drug

gives the weight of dried drug

b) Subtract: weight of dried drug

Minus

weight of dried insoluble residue

gives the weight of alcohol soluble extractive

c) Alcohol extractive: = X per cent

$$= \frac{\text{weight of alcohol soluble extractive}}{\text{weight of sample taken}} \times 100$$

b) Diluted alcohol extractive. The diluted alcohol extract was a clear reddish brown liquid with a greenish tinge. It is stable upon long standing. When evaporated to dryness on a steam bath and dried in the oven, a smooth mass with lustre, rather brittle in consistence was obtained; it was dark reddish brown in color. The samples collected in June yielded a lower percentage of extractive than the October collection, with an average yield of 18.042 per cent and 22.6786 per cent respectively. The temperature of the oven was sometimes difficult to control at 110°C. Possible loss in weight of extractive could be due to temperature above 110°C which resulted in a possible alteration in the composition of the extractive.

Formula:

$$\text{Per cent of dilute alcohol extractive} = \frac{\text{Weight of aliquot extractive} \times \frac{\text{total mls. alc. extract}}{\text{aliquot mls. alc. extract}}}{\text{Weight of sample}} \times 100$$

c. Ether-soluble extractive. The determination of the ether extractive include an approximation of the volatile ether-soluble and non-volatile ether-soluble portions. Absolute ether is employed in this extraction since ether (ordinary) which contains small amounts of water dissolves some tannins, sugar, etc. (30, p. 374). By drying the total ether extractive (previously dried over sulfuric acid) in the oven at 110°C, the volatile portion (volatile oil content) is volatilized and is determined based on the loss in weight after drying to constant weight. The residue left after drying to constant weight represents the non-volatile portion of the ether soluble extractive, possibly resinous matter, fats, and pigments. The total ether extractive was olive green in color. The non-volatile portion was yellowish mass with some greenish brown particulate mass. An average total ether extractive from the June collection was approximately 2.3 per cent and is lower than that of the October collection, 2.8 per cent. The average yield of

the volatile portion is approximately 11.67 per cent of the total ether extractive; non-volatile portion is 88.33 per cent of the total ether extractive. Basing on the original weight of the drug, the volatile portion is approximately 0.34 per cent, which is comparable to the percentage yield of volatile oil obtained by the steam bath volatilization method. (See Table 7, c and Table 8)

Formula:

a) Total ether extractive:

$$\text{Per cent} = \frac{\text{Wt. of total ether extractive}}{\text{Weight of sample}} \times 100$$

b) Volatile ether soluble portion of drug:

$$\text{Per cent} = \frac{\text{Loss in wt. of total ether extract}}{\text{Weight of sample}} \times 100$$

c) Volatile ether soluble portion of total ether extract:

$$\text{Per cent} = \frac{\text{Loss in wt. of total ether extractive}}{\text{Weight of total ether extract}} \times 100$$

d) Non-volatile ether soluble portion of drug:

$$\text{Per cent} = \frac{\text{Wt. of oven dried residue of total ether extr.}}{\text{Weight of sample}}$$

$$= \text{Quotient} \times 100$$

e) Non-volatile ether soluble portion of total ether extr.:

$$\text{Per cent} = \frac{\text{Weight of oven dried residue}}{\text{Weight of total ether extract}} \times 100$$

d) Petroleum ether extractive. In general, petroleum benzin is employed in the extraction of fats and

fatty oil constituents of plants. The residual benzin distillate (extract) was a pale green clear liquid. When evaporated to dryness, it yielded a yellowish-white, brown tinted unctuous mass, which on long standing in the dessicator, turned into a white mass. The benzin extractive obtained ranged from 1.7 per cent to 2.3 per cent. The sample from the June collection gave the higher percentage yield. (See Table 9)

Formula:

$$\text{Per cent of petroleum ether extractive} = \frac{\text{Weight of dessicated extractive}}{\text{Weight of sample}} \times 100$$

e) Water extractive. An orange-yellow solution with slight opalescence was obtained by macerating the drug with water overnight (57, pp. 779 - 780). This filtered liquid, when evaporated to dryness on a steam bath, and dried in the oven was reddish brown. (It includes water soluble plant constituents such as possibly glycosides, soluble saccharides, coloring matter, tannin, saponins, etc.) Approximately, an average of 16.3 per cent water-soluble extractive was obtained. (See Table 10)

5. The alkaloidal assay of Ephedra viridis Coville based on the chromatographic method by V. Dalal (and M. L. Kharana, 17, pp. 165 - 174).

With the growing interest and the development of chromatographic methods of isolating substances (in pure form), this method of assay of Ephedra by V. Dalal and M. L. Kharana was followed in the assay of samples of Ephedra viridis Coville obtained from southeastern Oregon, in June and October, 1953. The method is originally based on the U.S.P. method of assay of Belladonna leaf, modified by employing lime, besides ammonia, to liberate the alkaloid from the plant cells, and adsorbing the chloroform - isolated alkaloidal principles upon alumina chromatographic column and then eluting the column with alcohol (90 per cent), the latter (eluate) titrated with 0.1 N acid (sulfuric acid). The amount of acid taken up by the eluate indicates the amount of total alkaloids present in the samples, in terms of Ephedrine, using the factor 0.01651 of Ephedrine per ml. of 0.1 N H_2SO_4.

a) Extraction of alkaloid. The air-dried powder (# 40) of Ephedra was triturated with 20 per cent lime (2 Gms CaO for 10 Gms. of the powdered drug- # 40). The mixture was placed in an Erlenmeyer flask and shaken with 100 mls. of chloroform. To this was added 10 mls. of 10 per cent ammonia solution and the mixture set in the shaking machine at 30 minutes intervals for three hours after which it was allowed to stand overnight. The mixture was then transferred into a percolator using at

first 110 mls. of chloroform as mentruum, and using additional mls. of the same solvent until the drug was completely exhausted. Last portions of the percolate (4 mls. portions) were tested for the presence of alkaloid by Valser's reagent (57, p. 72, 941) to check the complete exhaustion of the drug.

The percolate was collected in a separatory funnel and washed free of traces of ammonia by shaking the extract in a gentle rotary motion with 10 mls. portions of distilled water until freed of ammonia.

b. The chromatographic adsorption of the alkaloid extract. The chloroform extract was passed through the column of activated alumina powder, prepared by packing a chromatographic tube (glass tube 20 cms. long with 1.5 cms. internal diameter and with one end drawn out to a much narrower internal diameter, 3 mms., and 4 cms. long) with cotton wool at the drawn end and then the tube was charged with 15 Gms. of the adsorbent (alumina), evenly packed and then thoroughly moistened with chloroform prior to the adsorption process. The charged tube was set on a filtering flask and the flow of the liquid was controlled at 50 to 60 drops per minute by gentle suction. After adsorbing the chloroform extract through the column, 25 additional mls. of chloroform was passed through to insure complete adsorption, even developing of a possible

chromatogram, and also to insure complete washing off of non-adsorbed material. Suction was applied to completely exhaust traces of chloroform from the column. The adsorption column was then eluted with 50 mls. of 90 per cent alcohol, in portions, applying gentle suction such that the flow of the eluate was controlled at the rate of 50 to 60 drops per minute. Last portion of the alcohol eluate was tested for complete exhaustion of the alkaloid (57, pp. 72, 941; 17, pp. 173-174) from the adsorbed column. This insured complete elution of the column.

c) Titration. To the alcohol eluate, 25 mls. of N / 10 sulfuric acid and 25 mls. distilled water were added and the excess of acid not taken up by the alkaloids present was titrated with N / 10 sodium hydroxide solution, using methyl red as indicator. The total alkaloids present is determined in terms of Ephedrine, by multiplying the number of mls. of acid taken up by the eluate with the ephedrine factor 0.01651. (See Table 11, a, b)

Formula:

Per cent of total alkaloids

$$\text{in terms of ephedrine} = \frac{\text{Mls. of acid} \times 0.01651}{\text{Weight of sample}} \times 100$$

d) Discussion. The Ephedra powder submitted for investigation was obtained as a mixture of fine fibrous and powdered material tending to form lumps. Sand mixed

into it (fine sand was only available) rendered the sample only slightly better mixed temporarily. Sharp surgical tweezers helped in disintegrating the lumpy material and better sampling. The fibrous nature of the sample rendered the trituration with lime also difficult and thus hardly an even mixture. However, thorough mixing of the drug with the lime was effected in the Erlenmeyer flask by means of a firm horn spatula. In this case, the drug was weighed directly into the flask and to it was added the corresponding amount of lime required. This saves as much of the materials needed in the process of extraction.

The choice of lime (17, pp. 165 - 167, 173 - 174; 29, pp. 817 - 820) in the extraction process over other alkali such as suggested by some of the investigators on Ephedra (8, pp. 189 - 194; 11, pp. 109 - 115; 28, pp. 271 - 272; 29, pp. 817 - 820; 35, pp. 67 - 70; 40, pp. 313 - 324; 44, pp. 290 - 294; 47, pp. 1034 - 1039), makes for easier extraction of the alkaloid contents besides eliminating much of the impurities such as starch and gummy material and yielding a cleaner chloroform extract (17, pp. 165 - 167), thus facilitating subsequent steps. Lime and ammonia liberate the alkaloid from the plant material.

The chloroform solvent extracts alkaloidal principles, pigments and other chloroform soluble plant constituents.

The extract was bright green clear liquid. Excessive washing of the chloroform extract with water probably effected the color change from bright green to greenish yellow, which obstructed the accurate reading of the end point of the titration. Traces of ammonia must be completely washed off by water since it reacts with the acid resulting in an inaccurate determination. Water extracts a considerable amount of the ephedrine alkaloids. The chloroform solvent adsorbed, passed through the column as a clear faintly yellowish liquid. When evaporated spontaneously, a yellowish white, fatty residue was obtained. The residue had a peculiar faint aroma.

The chromatographic column developed distinct fine layers of colors such as green, yellow and red, in the order they are stated from the top layer to the bottom. Upon elution with 90 per cent alcohol, the layers of colors became indistinct in some of the columns but retained in the others. The alcohol eluate was brilliant green and clear, although two sample eluates were faintly yellowish green. A change of color from green to yellow was noted upon long standing of the eluates. When treated with the acid, some of the eluates became turbid, other sample eluates became turbid only upon the addition of distilled water, the rest remained clear. The turbidity of the titrant and the yellowish coloration rendered the

reading of the end point of the titration difficult, with methyl red as the indicator.

e) Detection of alkaloid. A main portion of the chloroform extract was tested for the presence of alkaloid, using Valser's reagent (57, pp. 72, 941). Two samples yielded only very slight turbidity while the rest of the samples showed no turbidity. Only one sample of the alcohol eluates yielded only very slight turbidity with Valser's reagent. None of the main portions tested gave a detectable (naked eye) biuret reaction (34, pp. 36 - 38; 57, p. 206) for the presence of Ephedrine. Even a standard solution of Ephedrine (0.1 per cent) barely gave a distinct biuret reaction. (A colorimeter might have detected it). Since the plant material was limited, no extensive detection nor isolation of possible alkaloid content was attempted.

The samples of the June and October collections of Ephedra viridis Coville yielded relatively similar results in the alkaloidal assay, that is, from 0.02 per cent to 0.29 per cent alkaloid content in terms of ephedrine. This is not a significant amount to encourage its cultivation for commercial source of ephedrine.

Certain conditions could affect the alkaloidal assay process. Ephedrine volatilizes at low temperature (53, pp. 27 - 30) and gradually decomposes on exposure to light.

Cold extraction process is advisable and the assay process should not be delayed. Long standing of the chloroform extract or the alcohol eluate before subsequent steps may affect the accuracy of results. Excessive washing of the chloroform extract with distilled water should be avoided since water extracts a considerable amount of the alkaloid. The washing process should be done cautiously to avoid formation of emulsion between the chloroform extract and water. Nevertheless, traces of ammonia left in the extract would affect the accuracy of the results in subsequent steps. Also, it had been noted by some investigators of Ephedra that seasonal variations (10, pp. 647 - 651; 19, pp. 87 - 96, 337 - 344; 22, pp. 636 - 641; 35, pp. 67 - 70), different cultural conditions (4, pp. 171 - 173; 39, p. 40) and age of the plant (13, pp. 199 - 209; 39, p. 40) affect the alkaloidal contents of Ephedra; that best yields are obtained from samples collected during Fall season while low yields are obtained from samples collected during rainy season. The nature of the powdered material investigated rendered difficult accurate sampling. The air-drying of the plant under sudden variations of humidity and long exposure may have affected the low alkaloidal yield.

SUMMARY AND CONCLUSION

A species of Ephedra plant, obtained by G. A. Hagey from a lot under clearing, located in southeastern Oregon was submitted to the School of Pharmacy, Oregon State College, for phytochemical investigation, on the basis that the dwellers in that vicinity have been reported as using the plant in the treatment of certain diseases such as veneral diseases, kidney disturbances and other affections. Samples of the plant were identified and verified by Dr. Albert Steward, curator, Herbarium, Botany Department, Oregon State College, as Ephedra viridis Coville. It was compared with the Ephedra viridis Coville collected in the Herbarium and is easily characterized by the bright green colored, slender, erect, jointed, scabrous branches, in broom-like arrangement. The leaf scales are opposite and connate, 3 to 6 mms. long, with usually only the brown thickened base persistent.

The powdered, air-dried samples of Ephedra viridis Coville were submitted to the different methods (mostly official in the U. S. Pharmacopoeia) for plant analysis and yielded the following corresponding results (approximate results):

1) Moisture determination: (Table 1, a, b)
   a. Toluene distillation method - 7 per cent
   b. Dessicator method - 5.5 to 7 per cent

2) Ash determination: (Table 2, a, b, c)

   a. Total ash - June collection - 6.3036 per cent
      October " - 7.8741 " "

   b. Water soluble ash - 0.86116 per cent

   c. Alkalinity of water-soluble ash - 0.9318 (mls. 0.1 N HCl per gram sample)

   d. Water-insoluble ash - 7.1211 per cent

   e. Alkalinity of water-insoluble ash - 12.323 per cent

   f. Acid insoluble ash - 0.1583 per cent (June), 0.1056 per cent (Oct.)

3) Crude fiber content - Table 3

4) Volatile oil - 0.3 per cent to 2 per cent (steam-bath method - Table 4)

5) Extractives:

   a. Alcohol (95 per cent) soluble extractive - 33.2 per cent

   b. Diluted alcohol extractive - 18.042 per cent to 22.68 per cent

   c. Ether (absolute) extractive:

      1. Total ether extractive - 2.3 to 2.8 per cent

      2. Volatile portion (volatile oil) - 0.34 per cent

      3. Non-volatile portion - 2.5 per cent

   d. Petroleum ether extractive - 1.75 to 2.27 per cent

   e. Water extractive - 16.2957 per cent

6) Alkaloidal assay (V. Dalal chromat.)

a. 3 Determinations - 0.025 to 0.0389 per cent

b. 3 Determinations - 0.138 to 0.186 per cent

c. 2 Determinations - 0.2018 to 0.299 per cent

The insignificant alkaloidal content in this particular species of Ephedra does not give a basis for encouraging the cultivation of the plant. However, the medicinal use of the plant by the Indian tribes, in the treatment of various diseases should encourage further investigations on the plant and other species for other active principles; also the conditions affecting the yield in alkaloidal assay could be remedied as to produce better results. Otherwise, the medicinal use of the plant by the Indian tribes with regard to the therapeutic value of ephedrine appears questionable. (See Table 11)

Table 1
Moisture Determination
a. Dessication method

No.	Wt. of sample		Loss in weight		Per cent - H_2O	Remarks on samples
1)	2.3965	Gms. -	0.1360	Gm. -	5.6749	Collection-Oct., 1953
2)	2.3974	" -	0.1266	" -	5.2848	" " "
3)	2.3625	" -	0.1285	" -	5.4391	" " "
4)	2.4892	" -	0.1375	" -	5.5239	" " "
5)	2.2439	" -	0.1225	" -	5.4592	" " "
				Average yield =	5.4763	
6)	2.2194	" -	0.1545	Gm. -	6.9613	" June, 1953
7)	2.1384	" -	0.1496	" -	6.9956	" " "
8)	2.2346	" -	0.1547	" -	6.9229	" " "
9)	2.2579	" -	0.1557	" -	6.8957	" " "
				Average yield =	6.9438	

b. Toluene distillation method

	Wt. of sample -	Mls. H_2O collected	Per cent H_2O
1)	22.0335 Gms.	1.53 mls.	6.9439
2)	22.0609 "	1.55 ",	7.0260
		Average	6.98495 per cent

Table 2

Ash Determination
a. Total ash

No.	Wt. of sample		Wt. of residue		Per cent ash	Remarks
1)	2.3965	Gms.	0.18556	Gm.	7.7429	Samples Collect-
2)	2.3974	"	0.19356	"	8.0737	ed in Oct.,1953
3)	2.3625	"	0.18617	"	7.8771	
4)	2.4892	"	0.19326	"	7.7639	Nos. 1-5
5)	2.2439	"	0.17756	"	7.9130	
			Average total ash		7.8741 per cent	
6)	2.2194	"	0.13916	Gm.	6.2702	Samples Collect-
7)	2.1384	"	0.14596	"	6.8256	ed in June,1953
8)	2.2346	"	0.13526	"	6.0529	Nos. 6-9
9)	2.2579	"	0.13696	"	6.0658	
			Average total ash		6.3036 per cent	

Table 2

b. Water-soluble ash and alkalinity

No.	Wt. of sample	Wt. of soluble ash	Per cent	Mls. HCl (0.1 N)	Alkalinity (mls.acid per Gm sample)
1)	2.3965 Gms.	0.0164 Gm.	0.6843	1.62 mls.	0.676
2)	2.3974 "	0.0240 "	1.0011	2.4 "	1.001
3)	2.3625 "	0.0153 "	0.6476	2.61 "	1.05
4)	2.4892 "	0.0257 "	1.0325	1.9 "	0.763
5)	2.2439 "	0.0211 "	0.94032	2.5 "	1.114

Average:- Water-soluble ash = 0.86116 per cent; Aver. alkalinity = 0.9318

c. Water-insoluble ash and alkalinity

No.	Wt. of sample	Wt. of insol. ash	Per cent of ash	Mls. HCl (0.1 N)	Alkalinity (mls.acid per Gm drug)
1)	2.3965 Gms.	0.1696 Gm.	7.0586	30.95	12.91 mls.
2)	2.3974 "	0.16956 "	7.0726	27.45	11.03 "
3)	2.2439 "	0.17086 "	7.2322	28.24	13.03 "

Average per cent of water-insoluble ash - 7.1211

Average alkalinity of water-insoluble ash- 12.323 mls.

d. Acid-insoluble ash

No.	Wt. of sample			Wt. of acid-insoluble ash			Per cent	Remarks
1)	2.2194	Gms.	-	0.00166	Gm.	-	0.0748	June, 1953
2)	2.2579	"	-	0.00546	"	-	0.2418	collection
								Nos. 1 - 2
				Average per cent of ash		-	0.1583	
3)	2.3625	"	-	0.00256	Gm.		0.1084	Oct.,1953
4)	2.4892	"	-	0.00256	"		0.1028	collection
				Average per cent ash		-	0.1056	

Table 3

Volatile Oil Determination
(By steam bath volatilization)

No.	Wt. of sample	Loss in wt. Due to oil	Per cent of volatile oil	
1)	2.3965 Gms.	0.0565 Gm.	2.3576	October, 1953
2)	2.3974 "	0.0093 Gm.	0.3879	Collection
3)	2.3625 "	0.0093 "	0.3936	Nos. 1 - 5
4)	2.4892 "	0.0073 "	0.2932	
5)	2.2439 "	0.0089 "	0.3922	
		Average per cent - 0.7649		
6)	2.2194 "	0.0112 Gm.	0.5046	June, 1953
7)	2.1384 "	0.0095 "	0.4442	Collection
8)	2.2346 "	0.0250 "	1.1187	Nos. 6 - 9
9)	2.2579 "	0.0130 "	0.5757	
		Average per cent - 0.6608		

Table 4
Crude Fiber Content

No.	Wt. of sample	Residue	Ash	Crude fiber	Per cent
1)	3.1010 Gms.	0.5564 Gm.	0.0048 Gm.	0.5516 Gm.	17.7877
2)	2.9846 "	0.5061 "	0.0057 "	0.5004 "	16.76606
			Average per cent -		17.2769

Table 5
Alcohol-Soluble Extractive

No.	Wt. of sample	Residue in thimble	Alcohol extract.	Per cent
1)	2.2566 Gms.	1.3581 Gms.	0.7409 Gm.	32.8325
2)	2.3347 Gms.	1.3881 "	0.7835 "	33.5589
		Average per cent -		33.1957

Table 6
Diluted Alcohol Extractive

No.	Wt. Sample	Dil.Alc. Extract. (total)	Per cent of Dil. Alc. Extract.	
1)	2.0064 Gms.	0.42988 Gm	21.4255	October 1953
2)	2.0177 "	0.4454 "	22.0779	Collection
3)	2.0127 "	0.4454 "	23.5326	Nos. 1 - 3
	Average -		22.678	
4)	2.0258 Gms.	0.3533 Gm	17.4424	June 1953
5)	2.003 "	0.3488 "	17.4138	Collection
6)	2.0353 "	0.3922 "	19.2698	Nos. 4 - 6
		"		
	Average		18.042	

Table 7
Ether-soluble extractive
a. Water Extractive

No.	Wt. of Sample	Total Ether Extractive	Per Cent of Total Ether Extr.
1)	2.9846 Gms.	0.0843 Gm.	2.82449
2)	3.1010 "	0.0914 "	2.6153
3)	2.7862 "	0.0795 "	2.8533

b. Volatile-portion of ether extractive

No.	Wt. Sample	Total Extract	Loss in Wt. (due to vol.oil)	Per cent of Vol. Oil
1)	2.9846 Gms.	0.0843 Gm.	0.0101 Gm.	0.3384
2)	3.1010 "	0.0914 "	0.0096 "	0.3367
3)	2.7862	0.0795 "	0.0089 "	0.3277
			Average -	0.33426

c. Non-volatile portion of ether extract

No.	Wt. of sample	Residue	Per cent of Non-volatile Portion
1)	2.9846 Gms.	0.0742 Gm.	2.49
2)	3.1010 "	0.0715 "	2.31
3)	2.7862 "	0.0706 "	2.69
		Average	2.496

Table 8
Volatile-ether extr.: volatile and non-volatile portion

No.	Wt. Sample	Per cent of Total Extr.	Per cent of Vol.port.(of total extr.)	Per cent of Non-vol.port. (of total ext.)
1)	2.9846 Gm.	2.82449	11.98	88.01
2)	3.1010 "	2.6153	11.83	88.16
3)	2.7862 "	2.8533	11.19	88.80

Table 9
Petroleum Ether Extractive

No.	Wt. Sample	Total extractive	Per cent of Petro/ether Extractive	
1)	2.4243 Gms.	0.0417 Gm.	1.7201	June 1953
2)	2.1380 "	0.0379 "	1.7726	collection
3)	2.2948 "	0.0403 "	1.7544	
		Average	1.7490	
4)	2.1045 "	0.0478 Gm.	2.2713	Oct., 1953
5)	2.5758 "	0.0569 "	2.2090	collection
		Average	2.2401	

Table 10
Water Extractive

No.	Wt. Sample	Total extractive	Per cent of Water Extractive
1)	2.5867 Gms.	0.3862 Gm.	14.9108
2)	2.8076 "	0.4648 "	16.5515
3)	2.3346 "	0.4078 "	17.4256
		Average	16.2959

6. The Alkaloidal Assay of Ephedra viridis Coville

Table 11

a. Chromatographic method by V. Dalal (17, pp. 173 - 174)

No.	Wt. Sample	mls. N/10	Per cent of Alkaloid	Remarks
1)	10.0649 Gms.	0.152 mls.	0.02543	June 1953 (col.)
2)	10.0633 "	0.1571 "	0.0250	Oct., 1953 "
3)	10.0611 "	0.2371 "	0.03891	" " "
4)	10.0380 "	1.1255 "	0.1851	June " "
5)	10.0636 "	1.0781 "	0.1768	Oct. " "
6)	12.1453 "	0.2839 "	0.0386	" " "
7)	12.3702 "	0.2753 "	0.0370	" " "
8)	10.0828 "	0.8769 "	0.1435	" " "
9)	10.0806 "	0.8430 "	0.1381	" " "
10)	10.8550 "	1.3274 "	0.2018	" " "
11)	11.7146 "	2.1228 "	0.2992	" " "

b. Blank titration on alcohol solvent (90 per cent)

1) 50 mls. alcohol (90 per cent) -- .0002 mls. acid

2) 50 " " " " " -- .0004 " "

Remarks: Negligible amounts

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