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A study of the constituents of the Laptotaenia multifida -Nutt

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A STUDY OF THE CONSTITUENTS OF THE LAPTOTAENIA MULTIFIDA-NUTT.

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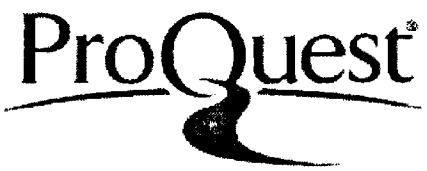
A thesis submitted in partial fulfillment of requirements for the degree of Master of Arts in Chemistry in the State University of Montana.



I N T R O D U C T I O N

The purpose of the work reported in this thesis has been the extraction and separation of the different compounds which might be found in the root of the plant known as Leptotaenia Multifida Nuttall.

Description of plant. Leptotaenia Multifida-Nuttall. Family Ammiaceae (Carrot Family) Fruit strongly flattened dorsally, with the lateral wings more or less prominently winged- Stylopodium wanting, plant acaulescent or nearly so. Lateral wings of the fruit thick, dorsal wings filiform. Tall and stout plants with three or four times compound leaves and involcules of small bractlets.(1)

This plant has many features which would lead one to expect that there might be some substances of importance among its constituents. It has been reported as the cause of death to cattle in many instances, although there has been no proof of the plant holding any poisonous compound. It belongs to a group of plants (Umbelliferae) which as a whole are considered to have strong physiological action.

Among the plants of the Umbelliferae family which are recognized as important in pharmacy are the following:(2)

1. Those with essential oils.

Plant	Oil
Carum carui	Oil of Caraway
Coriandrum sativum	Oil of Coriander
Anethum graveolens	Oil of Dill
Foeniculum Vulgare	Oil of Fennel

Cuminum Cyminum	Cumin Oil
Apium Graveolens	Oil of Celery
Ferula Narthex Ferula scorodosma)	Oil of Asafoetida
Ferula sumbul	Sumbul or Musk-root Oil.

Several cases of the death of cattle being ascribed to this plant a search of chemical and pharmaceutical literature was made but no record of any analytical work having been done on the Leptotaenia Multifida could be found. Work was then taken up to find some of the chief constituents of the plant through laboratory investigation.

Procedure.

A quantity of the root which was gathered the previous fall after a rather dry summer and stored in a dry place during the winter, was ground up by means of a food grinder. This broke up the structure of the root making it quite satisfactory for the succeeding procedures.

Moisture:

Three samples were taken for determination of moisture placed in a crucible weighed and then warmed in an oven the initial temperature of which was 95° C. This temperature was gradually increased until at the end of an hour it was 100° which temperature was held for an additional hour and a half. The crucibles were again weighed and the difference or loss in weight noted. Loss of weight divided by weight of sample gave percentage of moisture or what might more accurately be termed 100° volatil.

The following are the figures resulting: (weights in grams)

		I	II	III	
Before Heating	Weight crucible empty	9.6871	10.6523	9.1940	
	Weight crucible & sample	11.9615	12.5427	11.2960	
	Weight sample	2.2744	1.8904	2.1020	
After Heating	Weight crucible & sample	11.8065	12.4084	11.1483	
	Loss of weight due to Moisture etc.	.1550	.1343	.1477	
Percentage of Moisture:		6.82	7.10	7.03	
Average of accepted values:					7.07%

Ash:

The dried samples in the crucibles were then ignited with care-gently at first and later to dull red heat with the burner and

the residue when free from carbon weighed as ash.

The weights and values were:

	I	II	
Weight of crucible empty	9.6871	9.1940	
Weight of crucible & Ash	9.7800	9.2800	
Weight of Ash	.0929	.0860	
Weight of sample	2.2744	2.1020	
Percent of Ash	4.085	4.091	
Average of accepted values			4.088%

Nitrogen:

Nitrogen was determined by the Kjeldahl method(3) in which the nitrogen present is converted into ammonia which is distilled over and determined as such by absorbing the ammonia in a known amount of standard acid and then titrating to determine the amount of ammonia distilled over.

The following are the figures on the nitrogen determination:

Normality of hydrochloric acid .09994
Normality of sodium hydroxide sol'n. .11116 Ratio: $\frac{\text{N of NaOH}}{\text{N of HCl}} = 1.1122$

Weight of Sample of root	1.0007	1.0007	
Amount of acid in Receiving flask	100 Cc	100 cc	
Amount of sodium hydroxide required to neutralize	81.70 cc	81.80 cc	
Amount of acid neutralized by NH_3 from sample	9.132 cc	9.026 cc	
Nitrogen in sample	1.2617%	1.2765%	
Average of accepted values			1.269%

Protein present equals 6.37 x 1.269 or 8.084 %

Extractions:

Samples of the ground up root were then taken, placed in a Soxhlet extractor and treated with petroleum ether.

The following figures apply:

	I	II	III
Weight flask	25.7635	29.3606	22.2877
Weight flask & residue	26.2002	29.7755	22.6711
Weight extract	.4367	.4149	.3834
Weight sample	2.0019	2.0001	1.9988
Giving % of extract	21.82	20.78	19.64

Values #1 and #2 were accepted-Number three was considered unreliable due to a leaky cork.

Average percent of root soluble in petroleum ether 21.34

The insoluble portion of the above samples of root was then exhausted with water free ethyl ether by means of the same extractors.

	I	II	III
Weight flask	66.2356	44.1793	61.0354
Weight flask & extract	66.3068	44.2363	61.0968
Weight extract	.0712	.0571	.0614
Weight samples	2.0019	2.0001	1.9988
Giving % of extract	1.46	1.35	

Values one and two were accepted as the more reliable, particles of cork being evident in number three flask.

Average percentages for ethyl ether soluble 1.41

The extraction process was repeated using the root residue from the ethyl ether extraction. Absolute ethyl alcohol was used as the solvent.

Weight of flask	56.3112	63.4565	63.4446
Weight of flask & extract	56.9600	63.8658	64.0853
Weight of extract	.6488	.4093	.6407

All three caramelized slightly upon drying, #1 especially so and/had it to be discarded. The percentages of root soluble in ethyl alcohol after exhaustion with the previous solvents were #2 14.88 and #3 15.16.

Average of accepted values 15.02%

The next solvent used in this series of extractions was carbon tetrachloride but no solvent action was evident.

Extraction was then made with water as the solvent. The following results were obtained:

	I	II	III
Weight of flask	63.4440	61.0354	44.1796
Weight of flask and residue	65.3426	61.5590	45.0328
Weight of extract	.9652	.5236	.8532
Percent of sample	19.83	##	20.18

Average of accepted values 20.01%

This ended the series of extracts run on the small sample.

Crude Fiber:

A larger sample was taken and the amount of crude fibre determined by the Henneberge method. This method consists mainly in the extraction with dilute sulfuric acid and then washing with water the residue undissolved being acted upon by dilute sodium hydroxide. The fiber is then washed dried and weighed, weight of the residue divided by the weight of sample x 100 being the precent of crude fiber. The average of the accepted values for crude fiber was 13.46%.

A sample of root was then prepared which weighed 470.05 grams. It was placed in an extraction cylinder, made of tinned copper with a condenser cap for refluxing the solvent. Extracts were then made with the same kind of solvents as were used in the preliminary quantitative run in the Soxhlet extractors except that the carbon tetrachloride was ommitted.

After the water extract was finished on the large sample the root residue was placed in a large flask and treated with N/40 sodium hydroxide. Following this extraction the root residue was again washed with water and extraction made with 1% hydrochloric acid.

These extracts were then examined to see to what extent they could be separated into their several components.

Petroleum Ether Extract:

The first one attacked was the petroleum ether extract. This was an oily appearing, amber colored substance with an odor slightly resembling that of celery. Its specific gravity was taken. The two values found were as follows:

	I	II
Weight of bottle and extract at 20°	1.5919	1.4968
Weight of bottle alone	1.2604	1.1665
Weight of bottle full of water	1.5912	1.4965
Weight of water	.3308	.3281
Specific gravity 20° / 20°	1.0067	1.0044

Average of accepted values 1.0056

The extract was tested for glycerine and gave a slight trace.(4)

On attempting to make a fractional distillation the substance frothed, foaming up until it filled the vessel but gave no sign of breaking up. Attempts at distillation were then made under reduced pressure but the foaming was as pronounced as ever. Treatment with different solvents gave slight encouragement but no complete separation. Near the end of the research after much work on the saponification of the oil it was found that this extract upon hydolysis with sodium hydroxide solution broke up giving a mixture of volatil and non volatil acids and alcohols. Time did not permit of further investigation along this line.

Tabulated review of the solvents used in searching for a method of separating the petroleum ether extract into its constituents.

The extract was found to be insoluble in water, almost completely soluble in absolute alcohol, completely soluble in carbon tetrachloride. Acetone gave an emulsion which did not break down inside of three weeks, altho it seemed that there was a partial separation here it was impossible to prove conclusively. Absolute alcohol solution

of the extract when diluted with water gave a white emulsion-like precipitate which coalesced to form a substance seemingly identical with the original extract.

Then a method as recommended by Dragendorff[5] was followed.

Dried Extract

Treated with Absolute alcohol

Solution	Insoluble
tested with potassium iodide plus iodine reagent gave no test for alkaloids. This solution was then treated with a small amount of magnesium acetate which caused a precipitate to form. This was filtered off and more magnesium acetate solution added. This procedure gave repeated precipitates which were composed of the magnesium salts of the fatty acids present. Those of the highest number of carbon atoms in the chain coming down first. All the recovered acids were of a brown color and there was not enough of any to allow of purification and boiling point determination.	Small amount of dried crumbly substance which appeared to be soluble in alcohol but much more slow in going into solution than the other portion. This residue twice treated with water in which a small amount of sodium hydroxide had been dissolved.

Solution	Insoluble
Acidified with sulfuric acid gave yellow oily acid with a sp.g. of less than one. Filtrate from the mixture was treated barium hydroxide and) ammonium hydroxide gave white precipitate soluble in ethyl alcohol-volatil.	Brown plastic mass soluble in chloroform and in diethyl ether. A chloroform solution was made and an aqueous solution of sodium chloride added. This gave two layers and a precipitate.

Soluble in chloroform. This was further treated with sodium hydroxide solution, the solution being separated from the chloroform and acidified gave a brown volatil product too small in amount for identification.	Soluble in salt solution. Solution evaporated to dryness gave a white volatil product and a residue sodium chloride.	Insoluble. Dissolved in ether leaving the salt behind. Solution on evaporating left oily substance with pungent sickening odor.

Ethyl Ether Extract:

On the first large sample an extraction was made with diethyl ether and the extract dried. The extract was treated with distilled water ..... t gave an acid reaction, taste bitter.

Solution	Residue
gave no test for alkaloids. Chloroform extraction of aqueous solution gave a resin with bitter taste. Aqueous solution tested for tannin group acids gave negative reactions. Aqueous solution when treated with ether gave an extract on evaporation which was oily, colorless with an odor resembling that of green peas. Amount of extract too small for securing an amount of any of these constituents in large enough amounts for identification.	Completely soluble in absolute alcohol showing probable absence of acid resin.

Alcohol Extract:

The alcohol extract was next taken and the alcohol removed at 40° under reduced pressure until the residue became syrupy. This syrup was transferred to a small flask and the flask placed in a dessicator with a top fitted for a rubber stopper and the rest of the alcohol removed at room temperature by means of reduced pressure.

The pure extract was found to be slightly brown in color and powdery insoluble in diethyl ether, insoluble in chloroform and completely soluble in water.

Detection and Estimation of Tannin:

9.7108 grams of the extract were dissolved in exactly 200 cc. of distilled water. Each cc of this solution then represented .048554

gram of the extract. A small amount of this solution was tested for tannin by the use of solutions of ferrous and ferric salts and a strong indication of tannin resulted. Three samples, each 25 cc. of the solution, were taken and an excess of lead acetate was added. The precipitated tannate was then filtered off on weighed filter papers, dried and weighed. The dried precipitates were then incinerated and the amount of lead present in the precipitate determined. By subtracting the amount of lead oxide represented from the weight of the precipitate the weight of tannin present in the sample was determined and the percentage calculated.

The following weights and values apply to the tannin determination:

	I	II	III
Weight of papers	.5590	.5472	.5487
(two to a sample)	.6026	.5669	.6075
Total weight of papers	1.1416	1.1141	1.1152
Weight of paper and tannate	2.8138	2.8302	Discarded
Weight of tannate	1.6723	1.7161	
Weight of crucible	48.2613	50.4355	
Weight of crucible & lead	49.0730	51.2860	
Weight of lead oxide	.8117	.8505	
Weight of tannin	.8606	.8656	
Percentage of tannin	5.83	5.86	

Average of accepted values 5.845%

Determination of Glucose in Alcohol Extract:

27.285 grams of the dry extract were taken and dissolved in exactly 500 cc of water. 25cc of this solution were taken, the tannin precipitated by means of lead acetate and the filtrate treated with sulfuric acid until the lead was completely precipitated.(6) The filtrate was then diluted to 500cc and 25cc of this treated with an excess of Fehlings solution. The precipitated cuprous oxide filtered off, converted over to cupric oxide and weighed. From this data the

(7)

percentage of sugar present in the sample was determined.

Amount of extract present in 25 cc of solution	1.1369 gm.
Weight of crucible	7.9769
Weight of crucible and oxide	8.2021
Weight of cupric oxide	.2252
Percent of glucose in root soluble in alcohol	1.20

A similar run was made on another solution of the extract except that just before the use of the Fehling solution the sugar solution was boiled with 2 percent of acid under a reflux condenser for half an hour. The sugar was then determined as before. If any polysaccharides had been present this second determination would have been correspondingly higher than the first. The amount of extracted acted upon in this case was 1.3643 gm. The amount of cupric oxide formed was .2593. Percent of sugars, soluble in alcohol, present in the root 1.17. It was therefore evident that there was no polysaccharide present.

Average of the glucose determinations 1.185%

Sodium Hydroxide Extract:

The sodium hydroxide extract was made both on the larger samples and also on the smaller ones. The inconvenience of handling large samples in an accurate quantitative way led to the removal of a definite weight of the dried water exhausted residue and a quantitative extraction made on this by allowing N/40 sodium hydroxide to act on it for several days, filtering off the extract and washing the residue with distilled water to remove the last trace of alkali. The root residue was then dried at 100°C. and weighed. The loss in weight represented the amount removed by the solvent. From these figures the percentage of sodium hydroxide soluble could be calculated. The

verage for the first extract, which was made the summer of 1922 was .88%. Two samples were run on a different gathering of root in 1924 iving an average of 5.85%.

verage of all determinations of sodium hydroxide soluble 5.86%

ucilages and Albumin:

Mucilages and albumins were precipitated by acidifying the xtract and adding three volumes of 90% ethyl alcohol. The mixture as allowed to stand for 24 hours and the precipitate was filtered off. ne sample was run through in the summer of 1923 to find the amount of lbumin and mucilage precipitate. Owing to the trouble experienced n the drying of the precipitate the return on only one of the recipitates was considered reliable. The value obtained was 3.732% ncluding ash and 3.641% ash deducted. To check this two samples of he extract were acidified and the albumin and mucilage precipitated s before but to avoid the trouble experienced in the drying of the recipitate the filtrate was dried, weighed, the amount of sodium cetate formed in neutralizing calculated, deducted and by subtracting he precentage this remainder represented from 5.86% the percentage f albumin and mucilage was arrived at.

The figures and weights are:

	I	II
mount of extract taken as sample	30cc	30cc
eight of evaporating dish	48.2513	47.5422
eight of dish plus residue	48.3255	47.6180
eight of residue	.0742	.0758
mount of sodium acetate present	.0495	.0495
eight of extract residue	.0247	.0263
ample of original root represented by 30cc of extract was	7.0212	7.0212
ercent of root in sodium hydroxide extract not precipitated by alcohol	1.85	1.97

Average 1.91% 5.86% - 1.91% equals 3.95%

Average of all determinations of albumin and mucilage (Value includes ash) 3.84%

Aqueous Extract:

The aqueous extract was found to have a volume of 4083 cc. Albumin and mucilage combined were determined on this extract by taking three samples, two of 75cc and one of 50cc, acidifying with acetic acid and adding two volumes of absolute alcohol. The mixture was allowed to stand over night and the precipitate then filtered off onto weighed filter papers. These were then dried at 100° to 110° in the drying oven and then weighed. (8)

The figures are:	I	II	
Amount of sample represented	10.94 gm	10.94 gm	Discarded
Weight of filter paper	1.2935	1.3275	due to
Weight of paper and precipitate	1.5220	1.5545	poor
Weight of precipitate	.2295	.2270	filtration
Percent of albumin & mucilage	2.086	2.070	

Average of accepted values 2.078%

Determination of Dextrin etc. in Aqueous Extract:

Two samples of the water extract were taken, the volume of each being 25cc. The albumins and mucilages were precipitated with two volumes of alcohol and filtered off. The filtrates were evaporated to a small volume and the carbohydrates precipitated by the addition of four volumes of absolute alcohol. In this predipitation any dextrin, levulin or similar carbohydrates are thrown down. These were filtered off on weighed filter papers and dried at about 60°.

The following figures apply:

Amount of root represented in sample	3.65 gm.	3.65 gm.
Weight of filter papers	.5652	.5593
Weight of paper and carbohydrates	.6164	.6024
Weight of carbohydrates	.0512	.0431
Percent of carbohydrates	1.40	1.18

Saponin:

The filtrate from the precipitation of the sugars in the aqueous extract gave a heavy precipitate with barium hydroxide[9] indicating the presence of saponin which was accordingly estimated. Saponin, although present in the water solution in this extraction may not be estimated here as the total of the saponin may not be present.[9a] Two samples of the original root substance were taken and boiled with distilled water but the oil present made it impossible to be sure of the complete removal of the saponin- in fact it was found that the oil later prevented the complete precipitation of the saponin with barium hydroxide. In the next samples the oil was first removed by use of petroleum ether and the dried root residue digested by boiling water. The decoction was filtered and the solution evaporated to about ten cc. volume, diluted gradually with water until all was in solution. The albumins and mucilages were precipitated with alcohol and filtered off. The precipitate was treated with boiling 85% alcohol and filtered, the filtrate being added to that from the first alcohol precipitation. The combined filtrates were placed on the steam bath until free from alcohol, dissolved in a small amount of water and the saponin precipitated by the addition of hot saturated barium hydroxide solution. The precipitates were filtered off, washed with concentrated barium hydroxide solution until free from tannin, dried, weighed and ignited. The barium oxide present in the precipitate was calculated and subtracted from the weight of the saponin precipitate. From this the percent of saponin in the sample was calculated.

	I	II
Amount of sample	6.792 gm.	6.782 gm.
Weight of filter paper	.6840	.6406
Weight of paper and precipitate	.9080	.8850
Weight of precipitate	.2240	.2444
Weight of ash	.1588	.1856
Weight of saponin	.1350	.1404
Percentage of saponin	1.9876	2.0702

Average of accepted values 2.0089

The presence of saponin was confirmed in the filtrate from the sugar precipitation. This was done by extracting with chloroform and evaporating the solvent. (10a) A portion of the residue when shaken with water gave forth a decided froth. Another portion with concentrated sulfuric acid gave a red color. A third portion was treated with concentrated sulfuric acid and a bluish green fluorescence resulted.

Hydrochloric Acid Extract:

The dried root residue from the sodium hydroxide extraction was treated with 1% hydrochloric acid. The mixture was allowed to stand for two days and the extract filtered off. The filtrate was measured and a portion of the extract was neutralized with hydrochloric acid, evaporated to dryness in a weighed container and weighed again, in this manner sufficient data was secured to determine the percent of acid soluble.

	I	II
Amount of residue after extraction	78 gm.	
Volume of extract	1510 cc	
Amount of extract taken to neutralize	25 cc	
Weight of beaker	20.3888	25.0891
Weight of beaker and residue	20.3564	25.5665
Weight of residue	.4676	.4775
Weight of sodium chloride	.3430	.3430
Weight of dried extract	.1246	.1345

The weight of substance removed from the large sample was calculated and added to the 78 gm value of residue giving the weight of

the substance started with at the beginning of the acid extraction. The amount of root substance at the beginning of acid extraction was found to be 85.88 gm by determination # I and 86.12 gm. by #II. Using these figures the amount of original root substance was found and the percent of acid soluble calculated in terms of original root substance. The two determinations gave 9.19 and 9.43% acid soluble. The average of accepted values for acid soluble 9.31%

Determination of Starch:

Two hundred and fifty cubic centimeters of the acid mash were taken, neutralized with sodium hydroxide and treated, after boiling and cooling, with about .05 mg of diastase. The mixture was set in a warm place and the diastase allowed to act for four days. The diastase extract was then filtered off and sterile water and more diastase added and that allowed to act on the sample. This second diastase extract was filtered out and added to the first, the total volume of the extract and washings was 582 cc.

The residue from the extraction was dried and weighed, the weight being 6.4164 grams. Samples of the diastase extract were taken and evaporated to dryness. This weight less the weight of the sodium chloride formed in the neutralizing of the acid gave the weight of dissolved substance. From these figuresthe weight of the original root substance could be determined.

Weight of the residue from diastase extraction	6.4164
(Average of two determinations) soluble less NaCl	1.892
Weight of the sample taken	8.388

Sample represented 23.107 gm of the original root substance.

Two samples of 75 cc of the diastase extract were taken and 7.5 cc of 6 normal hydrochloric acid added.(11) The samples were

then boiled under a reflux condenser for two and a half hours. The samples were then cooled and their volume measured. Sample #I was 116.8 cc. Sample #II was 108.3 cc. Fifty cubic centimeters of each solution were taken and placed in Fehlings solution. The cuprous oxide was ~~reduced~~ oxidized to cupric and the amount of starch represented calculated:

	I	II
Weight of crucible	7.7222	8.6196
Weight of crucible and cupric oxide	7.7743	8.6740
Weight of cupric oxide	.0521	.0544
Starch represented by CuO	20.7 mg	21.42 mg.
Percent of starch	4.89	5.05

Average of accepted values 4.97%

Summary:

The following determinations were made, the percentage being based on the air dried root.

Moisture	7.07%	
Crude fiber	13.46%	
Ash	4.088%	
Kjeldahl nitrogen	1.269%	representing 8.084% protein
Petroleum ether extract	21.34%	Consisting of volatil fatty acids and alcohols. SpG of extract at 20° 1.0056
Ethyl ether extract	1.41%	
Absolute alcohol extract	15.02%	Tannins 5.845% Glucose 1.185% No polysaccharides.
Aqueous extract	20.01%	Albumin-mucilage precipitate 2.078% Dextrin etc., 1.29%
Sodium hydroxide extract	5.86%	Albumin-mucilage precipitate 3.84%
Hydrochloric acid extract	9.31%	
Saponin determination	.92%	
Starch	4.97%	Determined by diastase method.

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(9a) Dragendorf, Plant Analysis, page 67.

(10a) Dragendorf, Plant Analysis, page 68.

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