The Ethnobotany of Secondary Metabolism

Plant chemicals (aka phytochemicals) not involved directly in the growth and development of plants are called secondary plant chemicals or secondary plant metabolites. Examples include chemicals used to attract pollinators or seed dispersers and deter or repel herbivores. While the chemical and ecological aspects of these chemicals will be explored in lecture and again in advanced courses if you desire (e.g., plant physiology, plant biochemistry), today we will focus on the human side (ethnobotany) of these chemicals.

HEALTH NOTE: The exercises on coffee, tea, and chocolate today call for tasting. You have the option of not participating in the tasting, but please join others who are in order to discuss. These products contain caffeine. If you have peanut allergies, you will want to avoid the chocolate since it was made in a facility that also processes peanuts. For the chili-pepper exercise, you may want to wear latex or vinyl gloves (provided) or at the very least avoid touching your eyes during dissections and wash your hands with soap immediately after handling them to avoid chemical burns from the capsaicin.

I. Dye Plants

Natural dyes have been in use for thousands of years to color textiles, hair, or even skin. Biochemically, the dyes extracted from plants are typically secondary metabolites that aide the plant with various functions like pollinator attraction or defense. Depending on the plant species, dyes are obtained from the root, leaves, bark, seeds, or flowers. Two popular sources of natural dyes are walnut husks (e.g., the outer covering of the fruit of *Juglans nigra*, the black walnut) and turmeric (the dried and ground rhizomes of *Curcuma longa*, a relative of ginger).

The walnut fruits in the lab today were collected from around campus this past fall. The active dying agents in walnut husks are juglone (which oxidizes brown-black), plumbagin (a yellow pigment) and tannins, which oxidize dark brown and acts as a "mordant" in helping to bind the other compounds to the fabric. The juglone is known to have allelopathic properties (deters the growth of other nearby plants) and antiherbivore properties. Tannins, found in many different plants, have antiherbivory properties since they bind with protein in an herbivore's stomach and thereby decrease the nutritional value of the food to the consumer.

The turmeric powder can be purchased in any old supermarket, in the specie isle, but this powder was bought in bulk from an Indian store near the Lancaster train station. The dying agent in turmeric is curcumin, which has an earthy, slightly bitter and slight chili-like taste. Turmeric has antimicrobial activity and ecologically it thought provide a defense against microbial attack in the soil.

Today you were asked to bring a pre-washed white t-shirt for dyeing in either the black walnut OR the turmeric. Please choose just one dye, not both, and use just one shirt since we do not have room for two. While you may tie your shirt into knots to have a tie-dye effect, please only do so in one color to avoid a mess (shirts that hang out of the pots wick water up and out of pot and onto the hotplate and table – establishing an ELECTRICUTION HAZZARD and a mess).

Inspect the unprocessed walnut husks and turmeric powder.

Which color to you think each will dye your t-shirt?

Which one will you be using (choose only one)?

After the dyeing and rinsing process, which color did your shirt come out to be? Was it the same as your prediction?

Your instructor may have prepared the dye solution ahead of time in order to allow for time for the other exercises today. If that's the case, you only need to follow the procedure below. Otherwise, you will need to break off the husks of the walnuts if you will be dyeing with walnuts. Do not use a knife for this that you might accidently hurt yourself; rather, remove the husk by crushing with a piece of wood (e.g., a small piece of two-by-four). The recipe for both is as follows:

Walnut Dyeing

1. Add as much broken or ground walnut husk to a pot of water as you like (the more the darker your dye).
2. Bring to gentle boil and then reduce to simmer.
3. Mark your shirt tag with marker to indicate it is yours.
4. Wet and wring-out your shirt with water
5. Add shirt to liquid for 2 hours up to several days.
6. Afterwards, remove, rinse, and wash.

Turmeric Dyeing

1. Do the same as above, only add the turmeric powder and stir to dissolve before bringing to boil.

II. Stimulatory Plants

Coffee, tea, chocolate, and cola are four different species from three different families, yet they all have something in common: the alkaloid caffeine. Alkaloids are a class of psychoactive plant secondary metabolites affecting the central nervous systems (CNS) of the animals that ingest them. Some other notorious alkaloids are cocaine, morphine, nicotine, and quinine. Caffeine itself acts as a CNS stimulant and is mildly addictive. In addition to the four species discussed here, caffeine is found in at least 60 other plant species. Although people undoubtedly enjoy coffee, tea, chocolate and cola for their flavors, the caffeine has undoubtedly played a primary role in making these four species the most economically and socially important plants behind the major food crops.

A. Coffee (working in groups of 4)

1. Systematics. Grab a cup of coffee from the front/back/side of the room if you wish and answer the following questions. Use your class notes, books in the classroom, or the internet to answer.

What is the scientific name for the coffee species?

What is the scientific name of the family it is in?

What medicine used to treat malaria comes from this family? What is the species that this medicine comes from? What drink is flavored with this medicine?

To which country(s) is the coffee plant native?

2. Morphology. Examine the coffee plants in the room. We only recently received them and so they are still small seedlings. Then use the books in the room, your text, or the internet to answer some.

Looking at the living seedlings:

What is the phyllotaxy (alternate, opposite or whorled)?

What is the leaf venation (pinnnae, palmate, or parallel)?

Are the nodes stipulate or exstipulate?

Looking at the internet:

Is the fruit a berry or a capsule?

What color is the ripe (mature) fruit?

How many seeds does a typical fruit have in it?

Looking at the coffee beans:

A coffee fruit typically has two seeds in it, which are appressed tightly against one another and the central fruit septum.

Use this knowledge to explain why the seeds are typically flat on one side.

Find the longitudinal groove on a seed. This is the hilum. Look up hilum and define it below.

Why isn't the hilum on the round side of the seed?

Peaberries. A peaberry is a coffee seed that is rounded on all sides rather than be flat on the one side. It is from the occasional 1-seesded fruit. The coffee at your table has peaberries mixed with normal seeds (i.e., the naturally occurring peaberries on the trees at the plantation were not sorted out for special processing).

Find a peaberry and use this knowledge to explain how a fruit's 1-seededness influences the roundness of the seed.

Do you think peaberries taste any different than the flat-sided seeds?

Now determine the frequency with which peaberries form naturally by determine their frequency in the coffee you have. Each person should count 50 randomly drawn seeds and score them in a table you make below as "peaberry" or "flat-sided". Then, as a class we can tally all results for an accurate estimate of the frequency with which they form.

3. Roasting. Ripe, red fruits are harvested and the seeds are removed. Once cleaned, the clean seeds are placed in a plastic bag and allowed to ferment overnight. After slight fermentation, the seeds are spread across a surface and dried in the sun or at low temperature in oven or with lamp. The dried seeds ("green" coffee beans) can be stored in jar or in zip-locked bag until roasting. Coffee beans are roasted for 10-15 min at 200 ^{O}C (for light roasts) to 230 ^{O}C (for dark roasts), which is equivalent to 392-446^{O}F.

How does the appearance and smell compare between the green and roasted coffee beans at your table? Compare and contrast the appearance and smell of roasted and green (unroasted) coffee at your table.

Which smell nicer to you?

As a group of 4, place a fifth of a small Dixie cup's worth of green beans into a 4x4 inch piece of foil in a toaster oven in the back of the room that is preheated to 230 ^{O}C. **NOT THE CUP, ONLY THE SEEDS ON THE FOIL!** Roast for 10-15 minutes and then carefully remove the beans with a spoon onto a napkin or small plate.

Do these beans now smell better than when they were green?
What type of roast does this look like? (light, medium, or dark roast)

B. Tea (working in groups of 4)

Strictly speaking, tea comes from the leaves of just one species – *Camellia sinensis* – and it is this species that is the subject of our study in this section. In the general sense, however, "tea" may refer to any beverage made from plant material steeped in hot water. The "herbal" teas commonly sold in markets come from a variety of plants other than *Camellia sinensis* (e.g., mint leaves or rose hips) and these herbal teas typically naturally lack caffeine.

1. Systematics. Use the above paragraph, books in the classroom, or the internet to answer the following questions about the systematics of this species. .

What is the scientific name for the tea species?

What is the scientific name of the family it is in?

Name one other member of this family that is used in ornamental horticulture.

To which country(s) is the tea plant native?

2. Morphology. Tea plants are woody shrubs with gorgeous white flowers about 2 cm diameter and with many yellow stamens. It is the caffeine-rich leaves, however, for which this species is so valued. Have you ever seen a whole tea leaf? If there are some available in lab, study a whole leaf of tea and answer the following questions.

Is the leaf petiolate?

Is it simple or compound?

Is the blade margin entire, toothed, or lobed?

Is the venation parallel, pinnate, or palmate?

3. Black, Green, & Oolong Teas: Informational & Comparative Tasting Exercise. There are three major classes of tea: black (fermented), green (unfermented), and oolong (semifermented) (Ukers 1935: 453). These teas all come from the same species, but they are prepared differently following harvesting of the leaves.

Black tea processing: After harvesting, the fresh tea leaves are allowed to wilt to about 2/3 their original water content then, while still somewhat moist, they are typically crushed or torn in some way (e.g., the *CTC* or *Crush, Tear, Curl* method) and then allowed to fully oxidize. The crushing and tearing exposes the leaf cells to oxidation; as they oxidize, their color darkens – part of which is due to the breakdown of the chlorophyll during the oxidation process. The industry refers to this oxidizing step as "fermentation," although that is a misnomer since it does not at all resemble actual biological fermentation which, of course, occurs in the absence of oxygen! After oxidation, the leaves are dried and packed for sale.

Green tea processing: After harvesting, the leaves are not allowed to wilt or oxidize (i.e., no "fermentation"). Rather, they are killed by heating with steam or by baking, which prevents oxidation. The leaves are then dried and packaged for sale.

Oolong tea processing: After harvesting, the leaves are allowed to wilt to about 2/3 moisture then they are tumbled (e.g., in a basket) to promote bruising and oxidation. Oxidation is halted earlier than for black tea (e.g., 70%) by steaming or baking of the leaves (contrast to the 100% oxidation for black tea). After oxidation, the leaves are dried and packed for sale.

3a. Tea Beverage Study & Comparison. For this you will work in groups of 4 and need the following materials:

- 2 bags of black tea
- 2 bags of oolong tea
- 2 bags of green tea
- 1 two-cup aluminum sauce-pan
- 3 clean (food-grade) 200 ml glass beaker
- Popcorn or peanuts
- 16 small Dixie cups (4 per person: 1 for water to rinse your mouth, 3 for your tea samples).
- 1 hotplate

Directions: Boil one cup or 500 ml of water in the sauce pan. While you are waiting, put a fresh bag of black, oolong, and green teas separately into three separate, clean, small glass containers (beakers) label so as not to mix up tea types. Upon boiling:

Black tea – pour 1/3 cup (or 150 ml) into the black tea container and steep for 2 minutes.
Oolong tea – wait till water has cooled for 1 minute (to 80-85 °C), then pour 1/3 cup (or 150 ml) into the black tea container and steep for 2 minutes.

Green tea – after pouring the oolong water, do the same for the green tea and steep for 2 minutes.

After 2 minutes of steeping time for each tea, remove and discard the bags. Pour into paper Dixie cups, taste by slurping a little onto your tongue and record observations into Table 1. To cleanse your palate between teas, eat some popcorn or peanuts provided, or take a drink of water.

Table 1. Comparison of beverages made from tea leaves in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple teas.

	Green (Salada®)	**Oolong** (Bigelow®)	**Black** (Salada®)
Darkness (light; darker; darkest)			
Color			
Taste Body (weak; strong; strongest)			
Taste Sweetness (not sweet; semi-sweet; very sweet)			
Taste Bitterness (weak; somewhat bitter; very bitter)			
Taste Acidity (weak; somewhat acidic; very acidic)			
Even if you're not a tea fan, rank the three teas (0 = least; 1 = somewhat; 2 = most)			

After you are done tasting teas, feel free to make yet another tea but this time add milk and/or sugar. Does this improve the taste for you?

3b. Dry Tea Study & Comparison.

As a group of 4, take one tea bag each of the black, green, and oolong teas.

How are the tea bags constructed? Draw one below and answer the questions.

Are they simply one simple bag with a single central compartment in which all of the tea sits? If not, then how are they different?

How does this design facilitate a more rapid infusion during the tea-making process than a more simple bag design?

Open each bag and study the tea inside. Then record your observations in Table 2.

Table 2. Comparison of dried leaves available in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple teas.

	Green (Salada®)	**Oolong** (Bigelow®)	**Black** (Salada®)
Color (green, dk. brown w/ green tinge, or dk. brown to black w/ reddish tinge)			
Aroma Strength (weak; strong; strongest)			
Aroma Sweetness (not sweet; semi-sweet; very sweet)			
Aroma Bitterness (weak; somewhat bitter; very bitter)			
Even if you're not a tea fan, rank the three teas (0 = least; 1 = somewhat; 2 = most)			

C. Chocolate (working in groups of 4)

The Aztecs and Maya were the first people to use the seeds of the cacao tree to make an edible drink, only they made a drink out of it and did not make the confection "chocolate" as we know it. Like coffee and tea, chocolate has caffeine in it, as well as a similar alkaloid, theobromine, with similar effect on humans.

1. Systematics. Use books in the classroom, or the internet to answer the following questions about the systematics of this species. .

What is the scientific name for the chocolate species?

What is the scientific name of the family it is in?

Name one other member of this family that is used to make a drink that is rich in caffeine.

To which country(s) is the chocolate tree native?

2. Dark, Milk, & White Chocolates: Informational & Comparative Tasting Exercise. There are three major classes of chocolate confection: dark, milk, and white. Unsweetened Baking Chocolate (essentially "raw" chocolate) is not considered a "confection" since it has no sugar added. These all come from the same species and the same part (the seeds), but they are prepared differently (Simpson & Orgorzaly 1995: 458).

Bitter, Baking & Unsweetened Chocolate are all names for the solid form of chocolate liquor or raw chocolate.* It has no sugar or milk added and so can be considered "raw" chocolate. To produce it, the cacao seeds (called "cocoa beans" by English speakers in the chocolate industry) are fermented, dried, roasted and separated from their seed coats (called "shells" by those in the industry). The seeds are then ground into cocoa paste which is then melted to become chocolate liquor. The liquor is then cooled and molded into blocks of unsweetened chocolate.

*Note that some baking chocolate has had some of the cocoa butter (fat) removed to increase the shelf life since fats such as cocoa butter will spoil before the cocoa solids. Raw chocolate from which all of the cocoa butter has been removed is dry and, when powdered, is called cocoa powder.

Dark Chocolate is produced by adding sugar and typically some additional cocoa butter to unsweetened baking chocolate. It either completely lacks or has only a very small amount of milk products in it. There is no official definition of "dark chocolate," but in practice it is a confection with relatively high chocolate liquor content (e.g., 50-85%) and little to no milk.

Milk Chocolate is produced by adding sugar, additional cocoa butter, and milk powder, milk and/or condensed milk to unsweetened baking chocolate. Because of the high sugar and milk products content, milk chocolate necessarily has much lower percentage of chocolate liquor in it than dark chocolate.

White chocolate is a white or ivory-colored confection of cocoa butter (the chocolate liquor minus the cocoa solids), sugar, and milk. Note that cheap white chocolates substitute some or much of the cocoa butter with solid vegetable oils. White chocolate has no cocoa solids in it and so it does not look or taste

like chocolate in the classic sense. Additionally, white chocolate – since it does not contain cocoa solids- contains only trace or negligible amounts of theobromine and caffeine in it. Much on the market has vanilla flavoring added to it.

Chocolate Study & Comparison. As a group of 4, taste the various forms of chocolate at your table (take one small square of chocolate per person, per chocolate type). Then fill in Table 3 below.

Table 3. Comparison of chocolates available in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple teas.

	Bitter	**Dark**	**Milk**	**White**
Color (black, dk.-brown, brown, or white)				
Sweetness (not sweet; semi-sweet; very sweet)				
Bitterness (weak to none; somewhat bitter; very bitter)				
Even if you're not a chocolate fan, rank the types from 0-4 as follows (0 = least; 4 = most)				

Rank the four chocolate types above as to relative content of the stimulants caffeine and theobromine. Read the introductory material on chocolate above again for the information necessary to make this ranking.

III. Peppers vs. Pepper

A) The Capsicum Peppers: *Capsicum annuum* and related species (Solanaceae)

The Capsicum peppers were originally confused by Christopher Columbus for the highly sought after black pepper plants. The fruits were found to be as pungent as the black pepper from the Orient and thus helped to justify his journey west. The reality was that a New World species (*Capsicum annuum*) was discovered and brought back to Europe where it spread in popularity. The popularity of *C. annuum* is based in the presence of capsaicin (one of several different pungent alkaloids) which gives the fruit its biting taste. Capsaicin is concentrated in the placenta and seeds of the pepper fruit. Our current understanding of capsaicin is that it binds to the same receptors that interpret heat/pain, thus, providing the

burning sensation in our mouths following consumption. High levels of capsaicin on the skin can cause uncomfortable skin rashes/irritation and can even result in chemical burns.

The concentration of capsaicin varies greatly between the different plants. Heat ratings of the various peppers are known as Scoville Units in honor of Wilbur Scoville who developed a method to determine "hotness". Volunteers were fed extracts from a pepper and the sample was continuously diluted until the test subjects could no longer detect the pungency/heat. A pepper with a rating of 1 was diluted once (1:1) and a pepper with a rating of 100,000 was diluted to a final ratio of 1:100,000 (parts extract to parts water). Current uses for Capsicum pepper extracts range from consumption to the pepper spray used by police to arthritis treatments.

1. Two representative **Capsicum plants** are provided for your observations.
   Observe the living plant specimens provided and answer the questions below:
   a) *Capsicum annuum*
   b) *Capsicum sp.*

   Observe the morphology of the leaves:

   What type of venation is present in the leaves?

   The leaves have what type of complexity?

   Observe the morphology of the flowers (if present)

   Is the plant dicot of monocot based on the flowers?

   Why do you make this conclusion?

   Do the leaves support your expectations?

2. Various **fruit specimens** from *Capsicum annuum* have been provided for your analysis. Observe the provided specimens and answer the questions below:

   a) Bell pepper
   b) Miniature sweet bell pepper
   c) Guajillo pepper
   d) Habanero pepper
   e) Jalapeno pepper
   f) Long hot (cayenne) pepper
   g) Serrano
   h) Cubanella

Based on your pre-existing experience with peppers, try to identify the peppers and associate them with their appropriate Scoville Units (Choose from the provided list below, some ranges will be used more than once) Fill in the table:

Scoville Unit Ranges:
0
0-1,000
2,500-5,000
10,000-25,000
30,000-50,000
100,000-300,000

Identifier		Name	Scoville Units
A	Predicted:		
A	Actual:		
B	Predicted:		
B	Actual:		
C	Predicted:		
C	Actual:		
D	Predicted:		
D	Actual:		
E	Predicted:		
E	Actual:		
F	Predicted:		
F	Actual:		
G	Predicted:		
G	Actual:		
H	Predicted:		
H	Actual:		

Observe the external morphology of the bell pepper. Make a cross-section of the fruit and observe the internal anatomy:

What external structures are observable associated with the pericarp?

On what structure are the seeds located?

What type of placentation is present?

Draw what you see:

How many carpels were present in the ovary of the pistil?

How do you know?

Do the other fruits present vary from the bell pepper?

Image source: http://www.meemelink.com/prints%20pages/16746.Solanaceae%20-%20Capsicum%20annuum.htm

B) Black and White Pepper: *Piper nigrum* (Piperaceae)

Pepper comes from the *Piper nigrum* plant which is a flowering vine native to India and the East Indies. Peppercorns are intact fruits from *Piper nigrum*. Our first exposure is typically ground pepper which is derived from the peppercorns. Volatile oils (essential oils) give the distinct "biting" flavor associated with pepper. These oils dissipate after time which is why freshly ground pepper is typically preferred and also has a stronger taste to it.

1. Observe the provided specimens and note what botanical structure(s) represents our concept of pepper.

> **BLACK PEPPER**: Fruits (drupes) are picked green just before ripening and allowed to dry for a few days. During this drying period, the fruits turn black and shrivel.
>
> **WHITE PEPPER**: Fruits (drupes) are allowed to ripen on the plant and the outer hull is removed after harvest. The remaining peppercorn has a grayish color associated with it.
>
> **RED PEPPER**: Not *Piper nigrum* at all, actually derived from various Capsicum species (*C. annuum*). Red pepper flakes are processed by crushing/coarsely grinding the dried pepper fruit and typically includes whole seeds. Ground red pepper is ground dried fruit/seeds and is often referred to as Cayenne pepper and is also an ingredient of Chili powder (with several other ingredients).

What botanical structures were you able to identify in each of the specimens provided? Cross-reference with your answers for the table in the spices section

Black pepper =

White pepper =

Red Pepper =

Chili Powder =

2. Observe the label on the Paprika spice container.

What group of plants does the label suggest it comes from?

What botanical structure(s) would be used to produce Paprika?

IV. Flavor & Spice Plants

Spice plants (including herbs) have been used by humans for thousands of years to impart flavor, mask foul flavors, or to help preserve foods. Their function in the preservation of foods indicates that they often have antimicrobial activity and this is one of the functions they may carry out for their plants: preventing microbial/pathogen attack. Other roles may be to deter herbivory. Examine the various spices from the market. Use books in the room, the internet, and your powers of observation to complete the following informational table.

Table 4. Various spices and parts of the plant they come from. Place a check in the appropriate column/cell for each spice.

	Stem (e.g., bark)	Leaf	Flower (e.g., buds; stigmas)	Fruit	Seed
Bay laurel					
Chili (red pepper)					
Cinnamon					
Cloves					
Ginger					
Mace					
Nutmeg					
Oregano					
Paprika					
Pepper, black					
Pepper, white					
Star-anise					
Turmeric					

V. Review Questions & Exercises

1. What color did you predict the dye to set in your t-shirt?

2. Was this the color that you found after rinsing?

3. What are the scientific names of coffee, tea, and cacao?

4. What family is coffee in and what other medicinal plant is found in the family and how is it medicinal?

5. What other alkaloid stimulant does chocolate have in it that coffee and tea do not?

6. Coffee beans are actually what part of the plant (use the technical English term)?

7. To which continent and country(s) is coffee native? Why, then, is the scientific name for this species a bit misleading?

8. Which aroma did you find mind appealing, that of the green or roasted coffee beans?

9. After roasting your green coffee beans, did get beans that looked and smelled like something you were a little more familiar with?

10. Tally the results of the class for Table 1 on tea tasting. Summarize the class conclusions by assigning the numerical value to each descriptor (as in the left column below), summing them across each student for each cell and then taking the average to discover the average conclusions for the class.

Table 1.B. Class averages for comparison of beverages made from tea leaves in lab. Use the descriptors provided in the left-most column. Note that you can use the same descriptor for multiple teas.

	Green (Salada®)	**Oolong** (Bigelow®)	**Black** (Salada®)
Darkness (0 = light; 1 = darker; 2 = darkest)			
Color (no number values, just discuss as class)			
Taste Body (0 = weak; 1 = strong; 2 = strongest)			
Taste Sweetness (0 = not sweet; 1 = semi-sweet; 2 = very sweet)			
Taste Bitterness (0 = weak; 1 = somewhat bitter; 2 = very bitter)			
Taste Acidity (0 = weak; 1 = somewhat acidic; 2 = very acidic)			
Even if you're not a tea fan, rank the three teas (0 = least; 1 = somewhat; 2 = most)			

11. If there is time, discuss the answers to Table 4 (the spices) with the class.

VI. Literature Cited

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Ukers, W.H. 1935. All about tea, vol. II. The Tea and Coffee Trade Journal Company: New York, USA.

Coffee plant from Köhler (1887). Image processed by Thomas Schoepke at http://www.plant-pictures.de.

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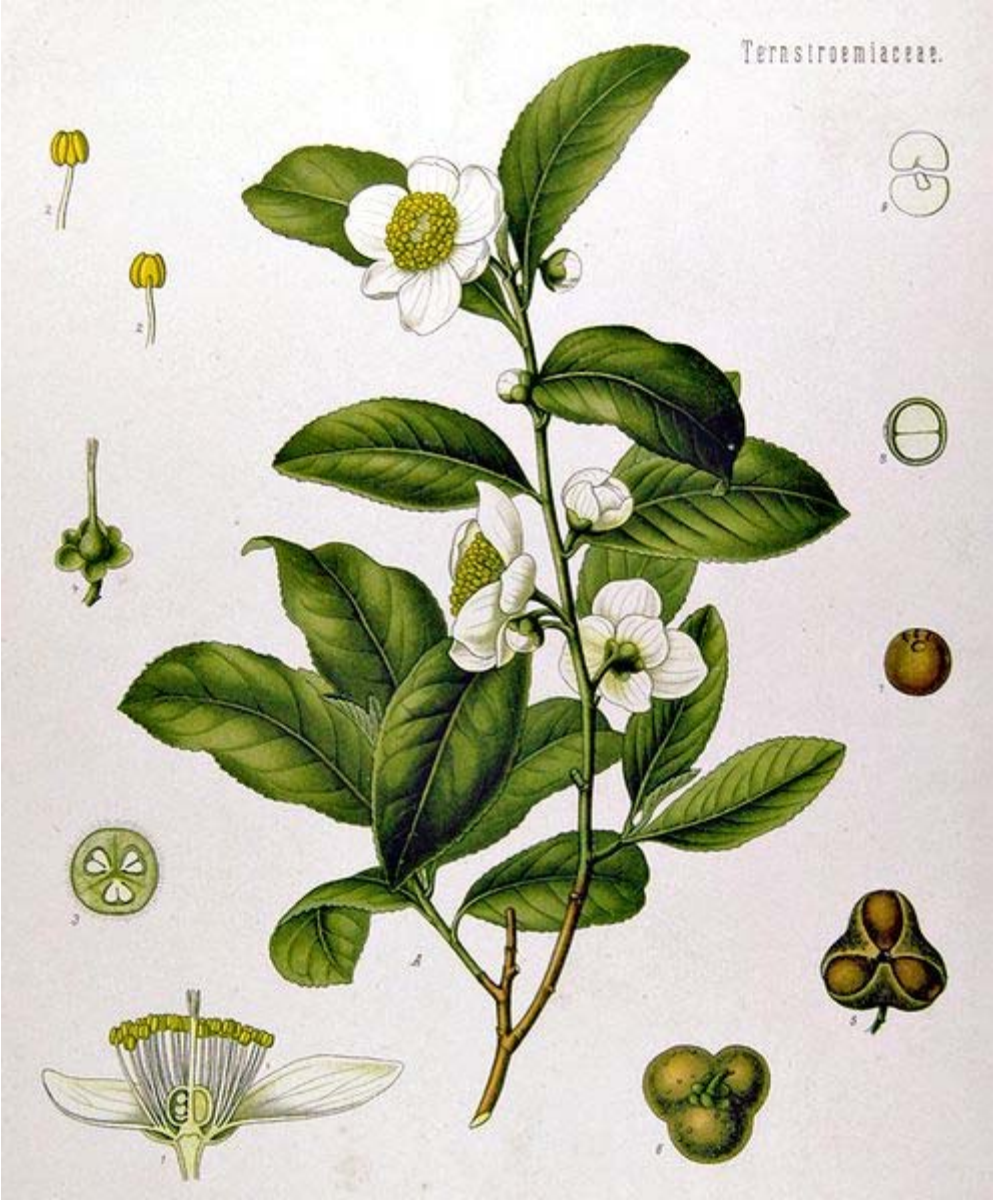

Tea plant from Köhler (1887). Image processed by Thomas Schoepke at http://www.plant-pictures.de.

PLATE XXXII.—*Theobroma cacao* (Chocolate tree). [From Jackson: *Experimental Pharmacology and Materia Medica.*]

Chocolate plant from www.wikipedia.org.

Piper nigrum
Image from: Wikipedia Commons - Koeh-107.jpg (466 × 595 pixels, file size: 168 KB, MIME type: image/jpeg)