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Abstract:

Atta cephalotes has shown potential as a bioassay tool for antifungal characteristics of medicinal plants (Hubbel, et al. 1983). This experiment tests a variety of plants with differing secondary compounds to explore further uses of *A. cephalotes* as a bioassay tool. A polar solvent was used to make crude extractions of twelve different medicinal plants to test the preference of *Atta* for the extract compared to a control. It was predicted that *A. cephalotes* would show a negative preference towards plants with antifungal compounds. However, negative preference for *Brugmansia suaveolens,* a plant with no known antifungal compounds, deterred *A. cephalotes* the most.

Resumen:

Atta cephalotes ha mostrado ser un potencial bio catador para plantas medicinales con propiedades fungicidas. Este proyecto es un prueba para plantas con componentes secundarios para explorar el uso de *A. cephalotes* como una herramienta bio catadora. Hice una extracción de doce plantas medicinales para probar preferencia de *A. cephalotes.* Se predijo que *A. cephalotes* tendría una negativa preferencia a plantas con fungicidas. Sin embargo, tuvo preferencia negativa para *Brugmansia suaveolens,* una planta sin fungicidas.

Introduction:

Analyzing plants and secondary compounds is a costly and time consuming endeavor. Bioprospectors must choose where to start, typically performing “modified random searches” (Lewis 1995). Leaf-cutter ants, *Atta cephalotes*, are pan-tropical and use leaves to grow their only food, fungus (Stevens, 1983). This makes leaf-cutter ants a potential bioassay tool, a crude first step in narrowing the search for potential medicinal plants. Studies have shown that leaf-cutters will avoid non-polar terpenoids more than polar compounds such as phenolics, alkaloids and glycosides (Howard 1987). Plants contain many combinations of secondary compounds and many have yet to be extensively studied (Hubert 1985), especially the synergistic effects of polar compounds.

Natural products and their derivatives, including antibiotics, represent more than 50% of all drugs in clinical use in the world (Wink and Wyk, 2004). Botanical medicine has been used by traditional cultures for centuries. Uncovering the mechanism by which phytochemical's act on the physiology of the human body has added scientific rational to many natural remedies. However, many traditionally used medicinal plants have yet to be extensively studied.

This study examines traditionally used medicinal plants of Costa Rica by observing the preference of *A. cephalotes* for polar secondary compounds. It is expected that *A. cephalotes* will show a negative preference for plants with known antifungal compounds. However, preference shown by leaf-cutter ants for plants high in alkaloids could represent plants worth researching further.

Methods:

Twelve plants were chosen to study based upon their medicinal qualities, growth in the Monteverde area (VanZandt 2002) and the recommendation of Lucas Villalobos, a medicinal plant expert at the Ecolodge in San Luis, Costa Rica. Recommendations were made about which plants were the most toxic and thus contained a wide variety and concentration of strong secondary compounds. Plant species used in this experiment were the following:

Family	*Genus and Specie*
Asclepiadaceae	*Asclepius curassavica*
Acanthaceae	*Justicia pectoralis*
Asteraceae	*Neurolaena lobata*
Asteraceae	*Chaptalia nutans*
Rutaceae	*Ruta chalepensis*
Solanaceae	*Brugmansia suaveolens*
Passifloraceae	*Passiflora biflora*
Simaroubaceae	*Quassia amara*
Solanaceae	*Solanum mammosum*
Euphorbiaceae	*Jatropha gossypifolia*
Apocynaceae	*Plumeria rubra*
Euphorbiaceae	*Chamaesyce hyssopifolia*

Crude secondary extractions were performed with a polar solution of 80% methanol and 1% hydrochloric acid mixed in a 1:1 ratio (Coley et al. 1989). 10 grams of plant leaves and 40 ml of solution were grinded for twenty minutes using the backside of a steel spoon upon a plastic plate. The plant extraction was then strained into a small vial to be carried to the leaf-cutter colony. Leaf picking, plant extraction and data collection were always performed within one day, ensuring a fresh extraction.

The leaf-cutter colony observed was located in San Luis, Costa Rica on the farm of Zaida Villalobos from April 15 to May 1 of 2007. The study site was at approximately 1100 meters in pre-montane wet secondary forest. Using forceps, oat flakes were dipped into the control, the polar solution, or the plant extraction and placed six inches from the entrance to the leaf-cutter nest. Two “control flakes” and two “extract flakes” were placed alternately on a large leaf-cutter trail, so that the lines of flakes were perpendicular to the trail. Respective oat flakes were replaced as soon as they were removed and recorded. Oat flakes were observed until they entered the leaf-cutter nest. Flakes were dropped or carried the wrong way very few times- these instances were recorded as well. Each plant extract was observed and recorded for one hour, generally in the afternoon.

A chi-square test was then run for each plant to determine the preference of *A. cephalotes* for each specific extraction.

Results:

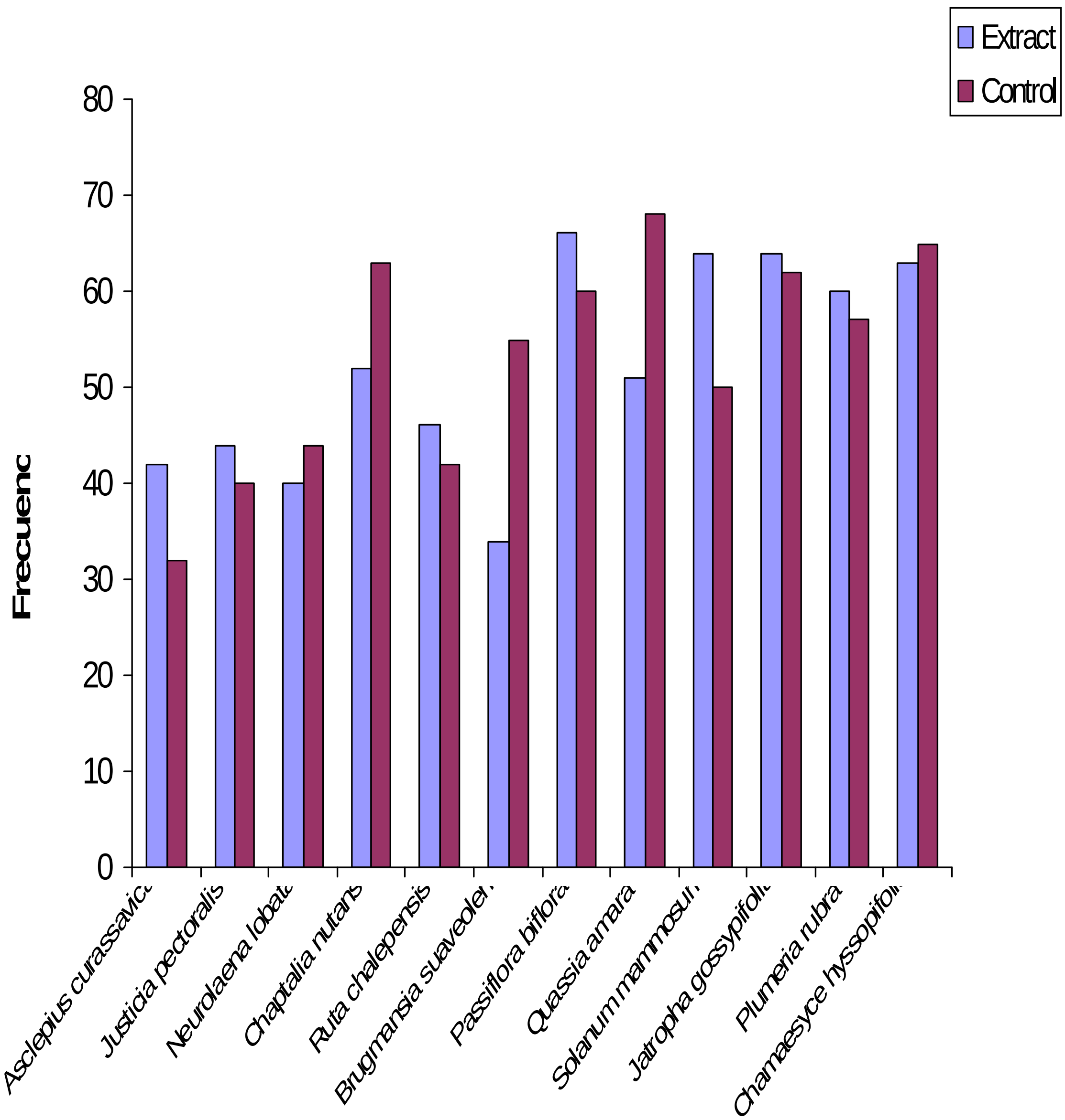

Figure 1. Frequency of selection for oats dipped in a polar extraction for selected plants compared to selection of the control (pure solvent) by *A. cephalotes*.

Atta showed the greatest preference in the case of *Brugmansia suaveolens* (X^2 = 4.955, p = 0.026, df = 1). In some cases *Atta* picked up more extract flakes than control flakes, as in *Asclepius* and *Solanum*.

TABLE 1: Chi-squared test's with calculated p – values for each plant.

Plant Species	p value	chi square	df
Asclepius curassavica	0.24504217	1.351351351	1
Justicia pectoralis	0.66252058	0.19047619	1
Neurolaena lobata	0.66252058	0.19047619	1
Chaptalia nutans	0.30500721	1.052173913	1
Ruta chalepensis	0.66981536	0.181818182	1
Brugmansia suaveolens	0.02601448	4.95505618	1
Passiflora biflora	0.5929801	0.285714286	1
Quassia amara	0.11914146	2.428571429	1
Solanum mammosum	0.18978349	1.719298246	1
Jatropha gossypifolia	0.8585862	0.031746032	1
Plumeria rubra	0.7815113	0.076923077	1
Chamaesyce hyssopifolia	0.8596838	0.03125	1

Brugmansia suaveolens showed the greatest preference between extract and control and represents the only trial statistically significant (p = .026, X^2 = 4.955).

TABLE 2: Additional observations: dropped or carried the wrong way. Plant species trials that never exhibited dropped or carried the wrong way behavior by *A. cephalotes* were omitted from this table.

Plant Species	Dropped (Extract)	Wrong Way (Extract)	Dropped (Control)	Wrong Way (Control)
Neurolaena lobata	0	1	0	0
Ruta chalepensis	0	0	0	1
Brugmansia suaveolens	1	8	0	1
Quassia amara	0	2	0	0
Solanum mammosum	0	1	1	1
Jatropha gossypifolia	0	1	0	0

In some rare cases *Atta* would drop the oat flake prior to bringing it into its nest. The oat flake was only recorded as dropped if *Atta* walked away from it after dropping it. However, every dropped flake was eventually picked up by another leaf-cutter and carried into the nest. In some cases *Atta* dropped the flake to get a better grip, this was not recorded. Another oddity observed was *Atta* carrying the oat flake away from the nest. This was only recorded if *Atta* walked against the traffic, away from the nest, for a distance greater than 10 cm. *Brugmansia* extract seemed to provoke this reaction more than other plants (Table 2).

Discussion:

These results were surprising. It was expected that *Atta* would show negative preference towards plants with known antifungal properties, such as *Plumeria rubra* (Hubbel, et al.

1983). Some of the plants are also listed as containing compounds known to be insecticides, as in *Quassia* and *Solanum* (Duke, 1994). It is interesting that the greatest negative preference was shown towards *Brugmansia.* *Brugmansia* contains an abundance of tropane alkaloids, such as scopolamine and atropine. These alkaloids are in the same class as cocaine and are strong antagonists at muscarinic acetylcholine receptors, inhibiting smooth muscle contraction. These compounds are also able to cross the blood-brain barrier, creating hallucinations (Wyk and Wink, 2004). *Brugmansia* also experienced a much higher proportion of cases where *A. cephalotes* would carry the flake the "wrong way" (Table 2). Perhaps these compounds are also able to upset the behavior of *A. cephalotes*. It is possible that the strength of tropane alkaloids and there high concentration in *Brugmansia* are capable of deterring *A. cephalotes.*

This is the first study to show that non-antifungal secondary compounds can deter *A. cephalotes.* It has been well documented that terpenoids contain the greatest antifungal activity (Hubert, 1985). In this experiment a highly polar solvent was used that is not effective in extracting the non-polar terpenoids. Perhaps there is more to the selectivity of substrate for fungal growth by *Atta* than is known. It would be interesting to isolate tropane alkaloids to see is they are the deterring factor or if it is a synergistic effect of many classes of compounds that determine the preference of *A. cephalotes*.

Minima were observed to be present in different concentrations on different days. On May 1, 2007 an extremely high abundance of minima were present. This day was different in that there was extremely dense cloud cover and the smell of ozone in the air. Perhaps *Atta* can sense biotic conditions such as air pressure and light intensity and change their behavior accordingly.

Some days there was more foraging behavior by *Atta* (Fig. 1). On day 1 they collected a total of 74 oat flakes and on day 12 they collected 128 oat flakes. Data collection commenced in mid-April and ended in the first week of May. Perhaps *Atta* can sense the onset of the rainy season and increases foraging behavior before the rains hit.
Plants produce varying amounts of secondary compounds at different times of the year, month and even day (Wyk and Wink 2004). The method of extraction used in this experiment was also very crude. For these reasons was difficult to tell whether a sufficient concentration of secondary compounds was obtained in each extraction.

Future studies should compare polar and non-polar secondary compounds by utilizing different extraction techniques and *Atta*'s preference towards them. There are also many more plants traditionally used as medicine that future studies could unveil using the same techniques described here.

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