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Adsorptive Capacity of Charcoals Eaten by Zanzibar Red Colobus Monkeys: Implications for Reducing Dietary Toxins

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Colobus monkeys on the African island of Zanzibar eat charcoal from burned trees and lying near kilns, where it is produced for cooking. This behavior may be a learned response for counteracting toxicity due to phenolic and similar compounds that occur in significant concentrations in the Indian almond (Terminalia catappa) *leaves and mango* (Mangifera indica) *leaves which constitute a major part of their diet. Accordingly, we studied the adsorption of organic materials from hot water extracts of Indian almond and mango leaves by five charcoals collected in Zanzibar. For comparison, we also evaluated three commercial powdered activated charcoals. Three African charcoals collected at kilns adsorbed more organic material than two kinds collected from burned tree stumps. The commercial activated charcoals adsorbed the organic material best, as expected, yet the African kiln charcoals adsorbed surprisingly well. Thus, the hypothesized function of charcoal eating is supported.*

KEY WORDS: *Procolobus kirkii*; charcoal; feeding; adsorption; phenolics

INTRODUCTION

Struhsaker observed Zanzibar red colobus monkeys (*Procolobus kirkii*) eating charcoal in areas where their home ranges include charcoal on a regular basis: in perennial gardens, in charcoal kilns, and near houses. They obtain charcoal from tree bases and stumps that are charred after pastures

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are burned, from active and abandoned charcoal kilns, and from bits accidentally dropped by people along paths (Struhsaker *et al.*, 1997).

Mturi (1991, 1993) also observed and reported charcoal eating by Zanzibar red colobus monkeys. She suggested that charcoal consumption may result in the "absorption" of gases and toxins from the gut. (The proper term for binding to charcoal is adsorption.) This habit may be analogous to the consumption of soil observed in several other colobine monkeys (Oates, 1978; Davies and Baillie, 1988). While a great many substances bind to charcoal *in vivo* (Cooney, 1995), it is doubtful that intestinal gases—H_2, CH_4, CO_2, and other species in lesser amounts—are among them, since simple gases are known to adsorb poorly to charcoal. Potter *et al.* (1985) observed no effect of charcoal on gas formation *in vitro* or *in vivo* with human subjects.

Mturi (1991) presented detailed analyses of 17 species of vegetation comprising the diet of the Zanzibar red colobus monkey, with values given for (1) weights of total phenolics as milligrams of tannic acid per gram of dry leaf, relative to *Trichoscypha patens*, (2) weights of condensed tannins as milligrams of quebracho tannin per gram of dry leaf, relative to *Trichoscypha patens*, (3) weight percent fiber cellulose and lignin by an acid detergent assay, (4) weight percent digestibility by a cellulase/pepsin assay, and (5) weight percent protein (dry basis). Results for two of the more important food species (*Terminalia catappa*, *Mangifera indica*) for some of the monkey groups are in Table I.

It is clear that both species contain significant amounts of phenolic materials. Phenolics, in such concentrations, could produce symptoms of toxicity, or at least interfere with digestive efficiency. Tannic acid, a polyphenolic, is known to cause severe gastroenteritis and abdominal pain, and to inhibit the absorption of substances from the colon (Arena, 1979), in overdose amounts. It is also a potent hepatotoxin. In addition, Hegnauer (1964) indicated that leaves of such species contain a variety of other potentially detrimental compounds, such as alkaloids, which, being predominately ring structures, also are known to adsorb well to activated charcoal. Thus, the eating of charcoal by colobus monkeys may be a behavioral adaptation that reduces or eliminates such toxicity by binding part of the phenolics to the charcoal, thus preventing their gastrointestinal absorption.

Table I. Analysis of Leaves in the Red Colobus Monkey Diet (Mturi, 1991)

Species	Leaf size and age	Total phenolics (mg/g)[a]	Condensed tannins (mg/g)[b]	Protein (% dry weight)
Terminalia catappa	Small, young	25.0	1.7	17.1
	Medium, young	23.6	0.3	15.1
Mangifera indica	Medium, young	49.4	0.9	17.9
	Full, young	20.0	0.6	14.8

[a]Compared to tannins in *Trichoscypha patens* (43 mg/g).
[b]Compared to quebracho tannin in *Trichoscypha patens* (49.8 mg/g).

Cooney (1995) discussed the effectiveness of powdered activated charcoal as an orally administered antidote for poisonings and drug overdoses. Powdered activated charcoal is now used widely throughout the world to suppress the gastrointestinal absorption of toxic substances in both humans and in domesticated animals.

Our objective was to determine the extent to which charcoals eaten by Zanzibar red colobus monkeys are capable of adsorbing compounds that are potentially deleterious to them.

MATERIALS AND METHODS

Struhsaker collected leaf and charcoal samples in 1994 from a study site next to the Jozani Forest, Zanzibar, Tanzania, where the red colobus fed on them (Struhsaker *et al.*, 1997).

We prepared extracts of dry Indian almond (*Terminalia catappa*) leaves and mango (*Mangifera indica*) leaves by adding 1.5 g of the leaves to 150 ml of distilled water in a 200-ml beaker, heating the water to boiling in a microwave oven, stirring the mixture for several minutes, and then filtering off the supernatant using a 50-ml plastic syringe connected to a filter holder containing a 25-mm-diameter, 0.45-μm-pore size millipore cellulose filter.

We obtained about 140 ml of extract in each case. The extracts were similar to brewed tea, and they had rich brown–yellow colors. We stored the extracts in a refrigerator, since we found that mold grew readily in the extracts if they were not refrigerated. Also, the extracts gradually darkened with time, due to oxidation of the organic materials (mostly phenolics). We refrigerated the extracts to greatly reduce the rate of oxidation. The considerable tendency of phenolics to undergo oxidation is described in detail by Taylor and Battersby (1967).

Whether the release of organics from dried leaves during a hot-water extraction process is similar to what occurs during digestion *in vivo* may be questioned. However, it seems reasonable that to a first approximation they are similar.

We ground the African charcoals (Table II) with a mortar and pestle and stored them in capped glass vials in order to simulate mastication by the monkeys.

Table II. Charcoal Samples Collected in Zanzibar, Tanzania, June–July 1994[a]

1. Sample 1: From a coconut palm stump. Struhsaker observed charcoal from this stump being eaten by red colobus monkeys.
2. Sample 2: From another coconut palm stump. Struhsaker also observed charcoal from this stump being eaten by red colobus monkeys.
3. Sample 3: "Annona" charcoal from a kiln.
4. Sample 4: "Mango" charcoal from a kiln.
5. Sample 5: Charcoal from an abandoned kiln where red colobus monkeys were observed eating charcoal by Struhsaker.

[a]Samples 1-4 collected at Jozani; sample 5 collected at Kiungani.

After grinding, we determined the internal surface areas of the five charcoals via the BET method, a standard procedure that involves the adsorption of nitrogen at its boiling point (−196°C). The surface areas were as follows: charcoal 1, near 0; charcoal 2, 3.2 m^2/g; charcoal 3, 299.5 m^2/g; charcoal 4, 306.8 m^2/g; and charcoal 5, 385.7 m^2/g. Thus, the charcoals produced by the slow heating of fresh wood in kilns in the absence of air (charcoals 3, 4, and 5) had fairly high surface areas, while those that resulted from the relatively fast charring of trees by wildfires had no significant surface area. These results are important, since the capacity of a charcoal for adsorbing an organic material is generally proportional to its surface area.

We also tested three commercial powdered charcoals obtained from the American Norit Company (Atlanta): Norit A, Norit B Supra, and Darco G-60. We kept them in their original sealed containers. Norit B Supra is made from coconut shells and has a high internal surface area, about 1400 m^2/g. Norit A is made from peat and has a surface area of about 720 m^2/g, while Darco G-60 is wood-based and has a surface area of about 600 m^2/g. One would expect the Norit B Supra charcoal to outperform the other two significantly, because of its very high surface area. This proved to be the case. The surface areas of charcoals 3–5 from Africa (about 300–400 m^2/g) are surprisingly high, considering that they were not purposely activated.

We carried out adsorption tests by adding 20 ml of the chosen extract (measured out by volumetric pipette) to glass vials into which predetermined amounts of charcoal had been weighed. We capped the vials and placed them in a shaker (100 cycles/min) for 24 hr. Then we filtered off the charcoal using 25-ml plastic syringes connected to filter holders containing 13-mm-diameter, 0.45-μm-pore size Millipore cellulose filters. We discarded the first few milliliters, which could be affected by the filters (through adsorption) or could contain membrane debris, and kept the next few milliliters in new glass vials. Then, we diluted 0.20 ml of each filtrate with 5–20 ml of distilled water to create final samples in a third set of new vials.

We scanned these solutions in a Perkin-Elmer Lambda 9 UV/visible spectrophotometer to produce graphs of the sample absorbance versus UV/visible radiation wavelength in nanometers (nm), over the wavelength range 200–400 nm. The transition point between the UV and the visible ranges in most spectrophotometers is around 345 nm. The absorbance (A) of a sample is given by $A = -\log(I_o/I_i)$, where I_o is the light intensity exiting the sample cell and I_i is the light intensity entering the sample cell. The absorbance increases with the concentration of the light-absorbing substance(s) in the sample solution. At lower values of absorbance, Beer's law is obeyed, and absorbance is exactly proportional to concentration. Thus, the absorbance becomes a direct and convenient measure of concentration.

We established the amount of distilled water to use in diluting the 0.20 ml of filtrate by the amount needed to reduce the maximum absorbances of the peaks of interest such that they fell within the limits of Beer's law. For the almond and mango extracts, we determined the linearity of absorbance versus concentration by scanning samples of 0.1, 0.2, 0.3, and 0.4 ml of extract diluted with 20 ml of distilled water. We found the relationships between peak absorbance values and concentration to be almost exactly linear for absorbances up to 1.50, and still close to linear up to 2.0, for each peak in the scans.

We recorded and printed out all sample UV/visible scans. The spectrophotometer also determined and printed out the positions in nanometers and heights in absorbance units of all peaks in the scans. Typical scans for the diluted almond and mango extract stocks are in Fig. 1. Of course, the resultant scans are the net product of the UV/visible absorbance characteristics and concentrations of hundreds, perhaps thousands, of individual organic species in the extracts. We did not attempt to determine the adsorption behavior of any individual species, or class of species, as this would have required an enormous amount of analysis, e.g., by GC/MS techniques. Thus, our scans are only a general index of what each extract contains, and how well the overall content of organics is lowered by adsorption to charcoal.

We divided the peak heights of the sample scans by the peak heights for samples of the stock solutions, diluted in the same way that the samples were. We assumed that these relative heights indicated how much organic material had been adsorbed by the charcoal, the amounts adsorbed being proportional to the differences between 1.0 and the relative peak height values. For example, a relative peak height of 0.82 would mean that a frac-

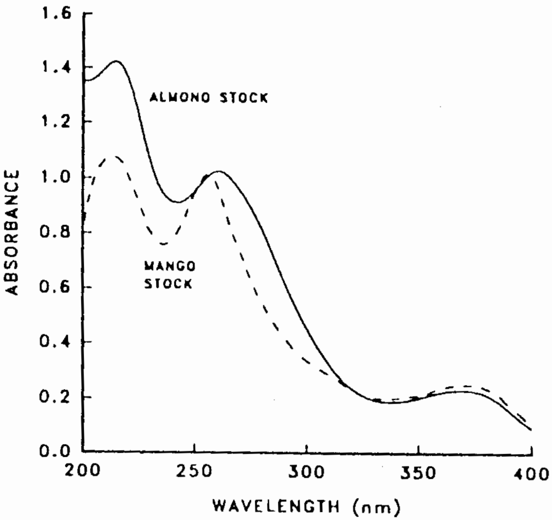

Fig. 1. UV/visible region absorbance scans of diluted almond and mango extracts.

tion = 1.0–0.82 = 0.18 (or 18%) of the organic material had been adsorbed by the charcoal.

RESULTS AND DISCUSSION

Figure 2 shows the relative peak heights determined in the almond extract adsorption tests. We prepared all samples using 0.50 g of charcoal and 20 ml of extract. For the scans, we diluted 0.20 ml of each filtrate with 20 ml of distilled water. Peaks occurred in the regions of 211–215, 257–261, and 361–368 nm in all scans. The peak heights for the diluted stock solution were 1.500, 1.067, and 0.269, respectively, in these three regions.

Figure 2 shows that charcoals 4 and 5 were the most effective in adsorbing organic material from the extract. This agrees with the fact that their surface areas are quite high. Why sample 3, which is also from a kiln

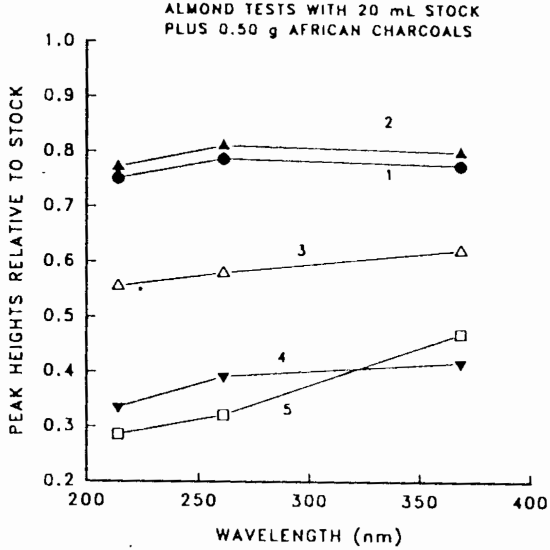

Fig. 2. Relative peak heights for the African charcoals and almond extract.

and has a surface area of comparable magnitude, is not as effective as samples 4 and 5 is unclear. It may be that some of the surface area determined by N_2 adsorption resides in small pores that are inaccessible to the adsorbable molecules in the almond extract. Charcoals 1 and 2, having low surface areas, performed less well, as expected, but not as poorly as one might have thought.

One can see that the amount adsorbed is a function of the wavelength. Each organic species in the extract would, of course, have a different scan and a different tendency to adsorb to charcoal. Thus, some variation with wavelength is to be expected. Which wavelength region is most representative of the organic material as a whole is not definite. However, the aromatic ring in phenolic compounds is responsible for the peak in the 257- to 261-nm region, so for phenolics that is the most representative peak. This is not to say that the effects of other structures such as aliphatic double bonds are insignificant, but only that the aromatic ring structures of phenolic compounds probably have the most important influence on the scans.

Figure 3 shows the relative peak heights determined in the mango extract adsorption tests. Again, all samples had 0.50 g of charcoal plus 20 ml of extract. We diluted 0.20 ml of each filtrate with 5 ml of distilled water. For the mango samples, peaks occurred in the regions of 206–213 and 256–259 nm. The peak heights for the diluted stock in those regions were 1.087 and 1.023, respectively. No distinct peak at high wavelengths (around 361–368 nm) occurred. Figure 3 shows that a relative peak height value greater than 1.0 occurred for charcoal 2 in the 206- to 213-nm region. This probably is due to the charcoal catalyzing significant oxidation of phenolics in the extract, even though contact was only 24 hr. As Cooney and Xi (1994) have demonstrated, the oxidation of phenolics when activated carbon is present as a catalyst can be relatively rapid. This converted some of the organic material to species that absorbed UV radiation more strongly in the 206- to 213-nm region. Thus, although the concentration of organics

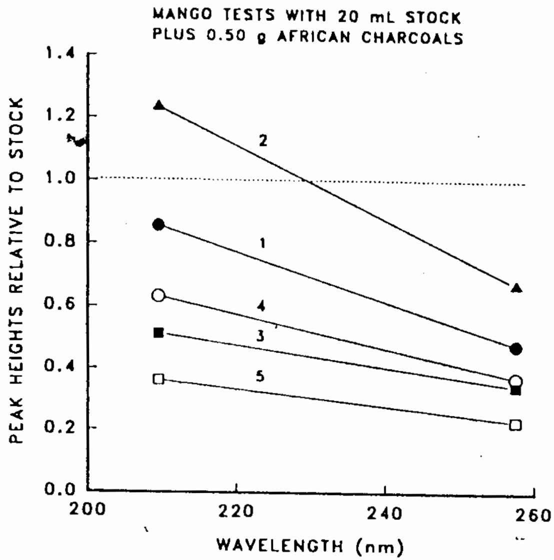

Fig. 3. Relative peak heights for the African charcoals and mango extract.

was probably lowered by the charcoal, their greater UV absorbance more than outweighed this. The data for the 256- to 259-nm region are probably less affected by the oxidation and are more representative of the adsorption effect. It is important to note the generally good adsorption of the mango extract components in the 256- to 259-nm region. Since the 256- to 259-nm peak is due mainly to the aromatic ring of phenolic compounds, then, if these are the more toxic compounds, their good adsorption is important physiologically.

The percentages of organic material adsorbed from the almond and mango extracts by the five African charcoals, based on the 256–261 nm peak height data are in Table III. We computed these values by subtracting the fractional relative peak heights, plotted in Figs. 2 and 3, from 1.0, and multiplying by 100 to give percentages. Charcoals 3–5, all of which came from kilns, adsorbed better than charcoals 1 and 2, which came from tree stumps. Moreover, a higher proportion of organic material was adsorbed from the mango extract. However, based on stock absorbances at equal dilutions, it appears that the mango extract had a significantly lower organic content than did the almond extract, so one cannot really draw firm conclusion in comparing the results for the two extracts.

We expected that the three commercial powdered activated charcoals would adsorb much more organic material, due to their much higher surface areas. Thus, we used smaller amounts (0.20 g) than in the case of the African charcoals (0.50 g). We kept the extract volume at 20 ml. Figure 4 shows the relative peak height results for the almond extract tests performed with the commercial charcoals. We diluted 0.20 ml of each filtrate with 20 ml of distilled water. Peaks occurred in the regions of 215–217, 261–263, and 363–367 nm. The peak heights for the diluted stock in those regions are 1.118, 0.800, and 0.193, respectively. Figure 4 shows that better adsorption occurred with 0.20 g of these charcoals than with 0.50 g of the African charcoals.

Table III. Percentage Organic Matter Adsorbed by African Charcoals[a]

	Almond extract	Mango extract
Charcoal 1	21	52
Charcoal 2	19	33
Charcoal 3	42	66
Charcoal 4	61	63
Charcoal 5	68	77

[a]Based on 256- to 261-nm peak height data.

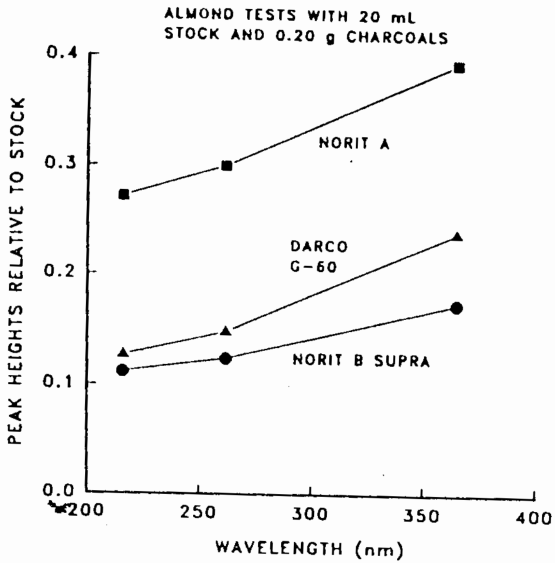

Fig. 4. Relative peak heights for three commercial powdered activated charcoals and almond extract.

Based on the peaks at 261–263 nm, adsorption is 88, 70, and 85% for the Norit B Supra, Norit A, and Darco G-60 charcoals, respectively, versus the Table III values of 21, 19, 42, 61, and 68% adsorption for African charcoals 1–5, respectively. This is unsurprising. If anything, the African charcoals performed much better than anticipated. The Darco G-60 charcoal is surprisingly effective, inasmuch as its surface area is less than that of Norit A. The Darco G-60 charcoal probably caused oxidative polymerization of the phenolics in the extract, as demonstrated by a very dark color in the filtrate from the Darco G-60 sample. Even though contact was only 24 hr, it is clear that oxidative coupling, which produces very highly adsorbable polymeric derivatives (dimers, trimers, etc.), occurred.

Table IV. Peak Height Ratios: Commercial Charcoals and Mango Extract

Sample	Peak wavelength region		
	201–208 nm	258 nm	296–312 nm
Norit B Supra	0.018	—	0.029
Norit A	0.093	—	—
Darco G-60	0.079	0.063	—

Table IV lists results with 0.20 g charcoal plus 20 ml mango extract. We used 20 ml of distilled water to dilute 0.20 ml of each filtrate. Peaks occurred over 201–208 nm in all scans but, at 258 nm and over 296–312 nm, in only two of the sample scans. The diluted stock absorbances in the three regions are 0.959, 0.461, and 0.315, respectively. The data show that the percentage adsorption is about 91–94% for Norit A and Darco G-60 and of the order of 97–98% for Norit B Supra.

CONCLUSIONS

If one takes the percentage adsorbed values for 0.50 g of the African charcoals and multiplies them by 0.20/0.50 to compare them with the results obtained using 0.20 g of the commercial charcoals, one obtains the following. For African charcoals 1–5 and almond extract, the percentage adsorbed values are 8.5, 7.6, 16.8, 24.4, and 27.2%, respectively. Norit A charcoal (0.20 g) adsorbed about 70.0% in the almond extract case. These results suggest that African charcoals 1 and 2 adsorbed about 11–12% as well as Norit A charcoal, African charcoal 3 adsorbed about 24% as well as Norit A charcoal, and African charcoals 4 and 5 performed about 35–39% as well as Norit A.

For the mango extract, the percentage adsorbed values for the African charcoals are 20.9, 13.1, 26.3, 25.2, and 30.8%, respectively, for an average of 23.3%. Norit A adsorbed about 91% in the mango extract case. Hence, the average for the African charcoals is about 26% of the value for Norit A. Nevertheless, the African charcoals performed better than expected.

Since the African charcoals adsorb significant amounts of organic material from both kinds of extracts, it is possible that eating charcoals significantly counteracts the toxic effects of compounds in the Zanzibar red colobus diet.

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Male Social Behavior and Dominance Hierarchy in the Sulawesi Crested Black Macaque (*Macaca nigra*)

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In a 6-week study of the social behavior of wild Sulawesi crested black macaques (Macaca nigra), *we found a linear and transitive dominance hierarchy among the six adult males in one social group. Dominance rank, as determined by the direction of supplantations, correlated strongly with percentage of time near more than four neighbors, frequency of grooming received from adult females, and percentage of time with an adult female as nearest neighbor. These results suggest that high-ranking males are socially attractive. Adult females sexually solicited high-ranking males more often than low-ranking males, but frequency of copulation was not correlated with dominance rank. Frequency and intensity of aggression between males are strongly correlated with rank distance, but aggression toward females was greatest for mid-ranking males. Males of all rank displayed significantly more aggression toward sexually receptive females than toward females in other estrous states. These data indicate that male Sulawesi crested black macaques display a social organization similar to that reported for multimale groups in other macaque species rather than the egalitarian social organization described for female Sulawesi macaques.*

KEY WORDS: Sulawesi crested black macaque; male dominance hierarchy; social organization; *Macaca nigra*.

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