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Research Article

In Vitro Antibacterial Potential of Extracts of *Sterculia africana*, *Acacia sieberiana*, and *Cassia abbreviata* ssp. *abbreviata* Used by Yellow Baboons *(Papio cynocephalus)* for Possible Self-Medication in Mikumi National Park, Tanzania

Irene Kirabo,[1,2] **Faith P. Mabiki**,[3] **Robinson H. Mdegela**,[2] **and Christopher J. D. Obbo**[4]

[1]*Natural Chemotherapeutics Research Institute, Ministry of Health, Kampala, Uganda*
[2]*Faculty of Veterinary Medicine, Sokoine University of Agriculture, Morogoro, Tanzania*
[3]*Department of Chemistry and Physics, Solomon Mahlangu College of Science and Education, Sokoine University of Agriculture, P.O. Box 3038, Morogoro, Tanzania*
[4]*Department of Biological Sciences, Kyambogo University, P.O. Box 1, Kampala, Uganda*

Correspondence should be addressed to Irene Kirabo; kirabokiraboz@gmail.com

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In the animals in general and nonhuman primates in particular self-medication has been widely reported; however, little is still known about the pharmacological activity of the extracts present in their daily diet. The *in vitro* antibacterial activity of the stem, root bark, and leaf extracts of three selected plants on which yellow baboons feed in an unusual manner in Mikumi National Park, Tanzania, was evaluated. Crude plant extracts were tested against Gram positive and Gram negative bacteria of medical and veterinary importance employing a modified agar well diffusion method and Minimum Inhibitory Concentration (MIC) technique. The lowest MIC value for Gram positive strains was 0.31 mg/ml demonstrated by *Cassia abbreviata* ssp. *abbreviata* against *Staphylococcus aureus* (ATCC 25923). The highest susceptibility to the ethanol plant extracts was exhibited by *Pseudomonas aeruginosa*, *Escherichia coli,* and *Staphylococcus aureus,* examples of microbes that affect both human and nonhuman primates. These findings demonstrate that the plant extracts from *Sterculia africana*, *Acacia sieberiana,* and *Cassia abbreviata* ssp. *abbreviata* have antibacterial activity and may be used as feed for their prophylactic benefits. Remarkably, the lowest MIC of 0.16 mg/ml was only 16-fold weaker than Gentamicin, a standard drug.

1. Introduction

There is a growing body of evidence to support the theory that animals are self-medicated by ingesting plants of both nutritional and medicinal value [1–5]. A Tanzanian medicine man, Babu Kalunde, almost a century ago, was able to treat a dysentery-like illness by observing a porcupine with similar symptoms ingesting the roots of *mulengelele*, a plant previously believed to be toxic [3]. Some of the evidence for self-medication by African great apes includes the infrequent intake of plant species which are not a regular part of the diet, restriction of plant use to seasons or other periods associated with high risk of parasitic infection [5–7], illness, or parasite infection of the individual at the time of ingestion of a putative medicinal plant [5, 6, 8], and a subsequent positive change in this condition following ingestion [7, 9, 10]. Furthermore, it has been suggested that compounds found in the ordinary diet of animals may have important positive effects on health and may prevent risks of infection and illness [2, 11]. Chemical investigations of the self-medication hypothesis have found that the bitter pith of *Vernonia amygdalina* has chemical compounds that apparently are responsible for the control of nematode infections [12]. *Diospyros abyssinica, Uvariopsis congensis, Albizia grandibracteata,* and *Trichilia rubescens* are only a few of the plants present in the nonprimate diet that have been tested for biological activity [13, 14].

Among the plants eaten by yellow baboons *(Papio cynocephalus)* inside Mikumi National Park, Tanzania, are *Acacia sieberiana* (Mimosaceae family), *Sterculia africana* (Sterculiaceae family), and *Cassia abbreviate* ssp. *abbreviata* (Caesalpiniaceae family), the focus of this study. The leaves and stem bark of *Acacia sieberiana* and the stem bark of *Cassia abbreviata* ssp. *abbreviata* and *Sterculia africana* were observed to be feed of the yellow baboons. On three separate occasions while observing yellow baboons, only one or two, as opposed to the whole group, would stop and feed on the leaves or stem bark of these plants and then join up with the rest of the group. A video of this phenomenon was recorded on one of these visits. However, though not observed to be consumed, the roots of all three plants and leaves of *Cassia abbreviata* ssp. *abbreviata* were analysed as well.

A number of studies have been conducted on *Cassia abbreviata* Oliv. [15], a close relative of C*assia abbreviata* ssp. *abbreviata*, endemic to Morogoro region from where this sample was taken [16, 17]; however there is a dearth of literature on *Cassia abbreviata* ssp. *abbreviata*. In Bukoba and Morogoro, Tanzania, the root bark of *Cassia abbreviata* ssp. *abbreviata* is used in the treatment of oral and vaginal candidiasis particularly in HIV/AIDS patients [18, 19].

Sterculia africana is valued in the study area for traditional worship and is one of the plants associated with ancestral sacrifices in Tanzania [20]. *Sterculia africana* root is used to treat asthma in communities around Lake Victoria region in Tanzania [21] and also possesses strong antifungal activity [18]. The Maale and Ari communities in Ethiopia utilize *Sterculia africana* as an antiemetic and to treat food poisoning as well as treating fever in the Blue Nile state, Sudan [22, 23]. *Sterculia africana* is one of the sources of nontraditional seed oils in Botswana [24] and is widely used in African traditional medicine. In Somalia a decoction of the crushed fresh root is drunk as an anthelmintic. In Tanzania a root decoction is taken to treat back pain, hernia, and dizziness, a root infusion is drunk as an aphrodisiac, and leaf decoctions are drunk for treating fungal infections and convulsions [25]. In parts of East Africa the roots, bark, and leaves are boiled and the vapor is inhaled for the treatment of influenza and fever. In Namibia a root or bark decoction is drunk by women for the treatment of postnatal and stomach pains, a leaf infusion is drunk for treating cough and chest complaints, and a fruit decoction is drunk to relieve pain during pregnancy and after giving birth. In Malawi the irritant hairs along the splitting point of the fruits are reported to be burnt and the ash is used in an ointment for the treatment of eye infections, especially in babies [26].

Acacia sieberiana has been utilized traditionally for treatment of skin eruptions and rheumatic pains and in treatment of syphilis, gastritis, cough, fever, ringworm, leprosy, epilepsy, dysentery, and mouth ulcers, as a vermicide and contraceptive [27]. Communities in South Africa and Ethiopia traditionally utilize *Acacia sieberiana* for the treatment of various ailments including inflammation, tiredness, joint pains, bilharzia, and fever and as an enema [28, 29]. The stem and root bark extract, both rich in tannins, are used in treating schistosomiasis, fever, stomach ache, jaundice, cough, sexual impotence, erectile dysfunction, haemorrhoids, syphilis, and uterine problems and to improve lactation after child birth [30]. Laboratory studies on *Acacia sieberiana* reveal that it is effective against *Bacillus subtilis, Staphylococcus epidermidis, Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae,* and *Mycobacterium avium*. Preliminary phytochemical screening showed presence of saponins, tannins, cardiac glycosides, flavonoids, and anthraquinones [31, 32]. The crushed pods are used in the treatment of hypertension in the Blue Nile state in Sudan [22].

This study was undertaken to establish the antibacterial activity of *Cassia abbreviata* ssp. *abbreviata*, *Acacia sieberiana*, and *Sterculia africana* against Gram positive and Gram negative bacteria of medical and veterinary concern. Results observed may contribute to knowledge on the range of microbes susceptible to these plant extracts using a single solvent.

Furthermore, results from this study may shed more light on the existing knowledge gap about the therapeutic benefits of plant metabolites present in the nonhuman primates' diet. A combined understanding of the underlying wildlife behaviour with long-term dietary data, antimicrobial studies, and analysis of plant chemistry will contribute to better understanding of the animal self-medication theory.

2. Materials and Methods

2.1. Plant Collection and Processing. Plant materials were collected from areas around Sokoine University of Agriculture main campus and areas around Mikumi National Park in Morogoro region, Tanzania, the location of which is shown in Figure 1. The samples were collected in the month of September during a dry spell when *Sterculia africana* had shed its leaves and therefore these were not studied.

The samples were collected during morning hours and authenticated with the help of Mr. Frank Mbago, a Botanist from the University of Dar es Salaam. They were assigned voucher specimen numbers FMM 3704, 3705, and 3706 for *Acacia sieberiana, Sterculia africana,* and *Cassia abbreviata* ssp. *abbreviata,* respectively. From *Sterculia africana,* the root bark and stem bark were collected while *Cassia abbreviata* ssp. *abbreviata* and *Acacia sieberiana* samples consisted of the stem bark, root bark, and leaves. The 8 samples were cleaned; the stem bark and root bark were cut into smaller pieces to allow for better drying in air. After drying, the samples were pulverised to a particle size of 1 mm.

2.2. Extraction of Samples. In the chemistry laboratory at Mazimbu Campus, Sokoine University of Agriculture, Tanzania, one sample was handled at a time in order to avoid cross contamination. Using a Yanhe Analytical Electronic Balance, the dry samples each were weighed into a separate clean, marked plastic container of known weight and the sample weight was recorded. The menstruum used was ethanol (Sigma-Aldrich) of analytical grade which was added till all the sample was fully soaked and macerated for 3 days with constant shaking. The samples were then strained and filtered using 110 mm Whatman filter paper and excess menstruum was evaporated using a rotary evaporator and then finally transferred to dry in an oven (Shel Lab) till constant weight.

Table 1: Codes of the extracts used during this study.

Name of plant	Code of plant	Plant part (p p)	Code of pp
Acacia sieberiana	A	Leaves	A.L
		Stem bark	A.S
		Root bark	A.R
Cassia abbreviata ssp. *abbreviata*	B	Leaves	B.L
		Stem bark	B.S
		Root bark	B.R
Sterculia africana	C	Stem bark	C.S
		Root bark	C.R



Figure 1: A map of Tanzania showing the location of Mikumi National Park and Morogoro region.

The marc was resoaked in fresh ethanol for 3 days and treated as the previous samples and then dried before being added to the corresponding samples. The crude plant extracts were then coded as shown in Table 1 and stored at room temperature before use for subsequent analysis.

2.3. Antibacterial Screening

2.3.1. Test Microorganisms. Agar well diffusion method was used for preliminary analysis to screen for samples with antibacterial activity and thereafter MIC was used for analysis.

Bacterial strains used in this study were standardized by the American Type Cell Collection (ATCC/Manassas, VA/USA) and constituted of Gram negative and Gram positive bacteria. The Gram negative bacteria tested were *Salmonella paratyphi* (ATCC 9150), *Klebsiella pneumoniae* (ATCC 13883), *Shigella sonnei* (ATCC 25931), *Enterobacter cloacae* (ATCC 23355), *Pseudomonas aeruginosa* (ATCC 27853), and *Escherichia coli* (ATCC 25922). Gram positive bacteria tested were *Enterococcus faecalis* (ATCC 51299) and *Staphylococcus aureus* (ATCC 25923). Bacterial culture cells were maintained at 37°C on Muller-Hinton (MH) agar on

Table 2: Minimum Inhibitory Concentration (mg/ml) of plant extracts.

Test organism	Code of plant part							
	C.R	C.S	A.R	A.S	A.L	B.R	B.S	B.L
E. coli (ATCC 25922)	1.25	1.25	1.25	0.63	0.31	0.63	0.63	0.31
S. paratyphi (ATCC 9150)	0.63	0.63	0.16	0.16	0.31	0.31	0.63	0.31
K. pneumoniae (ATCC 13883)	0.63	1.25	2.50	0.63	2.50	1.25	1.25	2.50
S. sonnei (ATCC 25931)	2.50	2.50	2.50	2.50	0.31	1.25	1.25	0.63
E. cloacae (ATCC 23355)	2.50	0.63	1.25	1.25	1.25	1.25	1.25	0.63
P. aeruginosa (ATCC 27853)	0.63	0.63	0.16	0.16	0.31	0.31	0.63	0.31
S. aureus (ATCC 25923)	0.63	0.63	0.63	0.63	0.63	0.31	0.63	0.31
E. faecalis (ATCC 51299)	0.63	1.25	2.50	0.63	2.50	1.25	1.25	2.50

slants until required. All microbial species used were supplied by the Department of Microbiology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania.

2.3.2. Minimum Inhibitory Concentration (MIC). The Minimum Inhibitory Concentration (MIC) is the lowest concentration of crude plant extract that inhibits growth of test microorganisms. A modified method by [33] was employed in determining the MIC of the different sample extracts. Sterile 100 μl of Muller-Hinton broth was measured in each well of a 96-well microtiter plate [33]. 100 μl of 10 mg/ml of extract prepared in dimethyl sulfoxide (DMSO) was added to row 1 and mixed using a micropipette. This was followed by serial dilution to row 8 with the additional 100 μl therein discarded. One column was used for sterility control (no culture was added), another column was used as a negative control (100 μl of DMSO without extract), and another column was used as the positive control with Gentamicin (0.01 mg/ml) making a total of 12 rows. 0.5 McFarland standard suspension of test bacteria was made in nutrient broth from which 100 μl of the final inoculums containing approximately 1×10^8 cfu/ml was added to each well except the sterility control to make a final volume of 200 μl. The experiments were performed in duplicate. The microtiter plates were sealed in a plastic film and then incubated at 37°C in a humidified incubator for 18 hrs. After incubation, 40 μl of 0.2 mg/ml iodonitrotetrazolium (INT) was added to each well and the microtiter plates were incubated for another 2 hours before removal for observation. The development of a purple colour resulting from the formation of the red/purple formazan was an indication of growth (positive indicator of cell viability). Decrease in the intensity of the red/purple formazan colour was indicative of inhibition of growth of test microorganisms.

3. Results

The MIC results tabulated in Table 2 showed that the crude plant extracts were active against all the test microorganisms. The lowest MIC value was 0.16 mg/ml as compared with 0.01 mg/ml of the standard, Gentamicin. The negative control showed development of a purple colour resulting from formation of the purple formazan which is a positive indicator of cell viability whereas the sterility control showed no colour change, an indication of absence of test microorganisms. Minimum Inhibitory Concentration ranged from 0.16 mg/ml to 2.5 mg/ml with 0.16 mg/ml being the lowest value denoting the greatest antibacterial efficacy as presented by A.R and A.S plant parts. Gram negative bacteria in the ethanol extracts had higher MIC values as opposed to the Gram positive bacteria. A.R and A.S demonstrated the lowest MIC values of 0.16 mg/ml and 0.31 mg/ml for both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* strains. The lowest MIC value for Gram positive strains was 0.31 mg/ml demonstrated by B.R and B.L against *Staphylococcus aureus*. Overall, plants A and B presented lower MIC values as opposed to plant C. The experiments were performed in duplicate and the average value was tabulated in Table 2.

4. Discussion

Cassia abbreviata ssp. *abbreviata*, *Acacia sieberiana,* and *Sterculia africana* extracts possess antibacterial activity against the test strains as shown from the results of the *in vitro* experiment in Table 2. *Cassia abbreviata* ssp. *abbreviata* and *Acacia sieberiana* exhibited better activity against the test microorganisms as compared to *Sterculia africana* with *Cassia abbreviata* ssp. *abbreviata* showing the lowest MIC value of 0.16 mg/ml of the three plants. The positive control was a standard drug, Gentamicin (0.01 mg/ml), an aminoglycoside targeting the bacterial ribosome. Interestingly, the lowest crude plant extract MIC of 0.16 mg/ml was only 16-fold weaker than Gentamicin. The crude plant extracts performed better than the negative control to a concentration of 0.313 mg/ml of extract. Other studies that reported on *Cassia abbreviata* ssp. *abbreviata* and *Acacia sieberiana* concur with these findings [16, 17, 30]. A viable challenge in interpreting self-medication is differentiating between plants that are ingested for their nutritional value but are medicinal foods and therefore offer passive prevention and plants ingested solely for their therapeutic medicinal use, medicinal plants. This challenge exists in traditional human societies where medicine and food are often of the same origin [2, 3, 28, 34]. For instance, traditional spices and condiments of daily Asian cuisine such as marine algae, ginger root, turmeric and herbs also play an important role in suppressing viral and parasite infections [2, 34, 35]. Results showed that different concentrations of the plant extracts were required to inhibit the growth of different microbes due to the difference in potency of the plant extracts attributed to the phytochemicals

present, environment of growth, or extraction method used. The variation in the susceptibility of microorganisms could also be attributed to their intrinsic properties that are related to the permeability of their cell surface to the plants extracts. Their pharmacological effect could therefore be experienced with repeated ingestion or work together in synergy.

This study provides evidence that the yellow baboon forages for similar plants also used in ethno medicine as seen from previous literature reviewed [16, 17, 22, 23]. 27.5% of microbes that affect nonhuman primates affect humans as well, *Staphylococcus aureus*, *Salmonella*, and *Escherichia coli* being only a few examples of such microbes, a factor attributed to our phylogenetic closeness [4, 12, 36, 37]. It should therefore not come as a surprise that human and nonhuman primates perhaps select similar plants when challenged with similar illnesses. It is known that nonhuman primates feed on a great variety of plant species; however it is prudent to take note of the ones that are used as feed infrequently and/or in isolated cases in an uncommon manner as was the case with the yellow baboons in Mikumi National Park. It may not be possible to pharmacologically analyse or carry out phytochemical screening for each plant; however, special attention should be paid to plants used as feed for nonhuman primates exhibiting potential symptoms of illness or in uncommon feeding behaviour [6–9, 11, 38] including but not limited to a lack of appetite, intestinal disorder, coughing, increased parasite load, adult parasite worm expulsion in the dung, and discoloration of urine.

5. Conclusion

The results of the *in vitro* experiment in Table 2 demonstrate that ethanol extracts from the different plant parts of *Cassia abbreviata* ssp. *abbreviata*, *Acacia sieberiana,* and *Sterculia africana* exhibit antibacterial activity against the test microbes. Some of these microorganisms are of medical and veterinary importance because they affect both humans and nonhuman primates. Additionally, these findings contribute to the foraging theory that suggests that the diet of nonhuman primates contains plants that are used as feed for self-medication. Their diet could therefore act as a sieve through which plants used as feed in an uncommon manner by nonhuman primates are tested. A combination of long-term dietary data, pharmacological studies, and analysis of plant chemistry may lead to the discovery of new therapeutic leads and also offer a better understanding of self-medication in animals. In addition to that, conservation of the plants used as feed is vital for primate conservation due to the pharmacological benefits obtained from these medicinal trees. These findings therefore propose the study of animal self-medication (zoopharmacognosy) as an alternative field in search for new therapeutic leads and drug discovery and further suggest that nonhuman primates feed on the nonnutritive parts of these plants for their therapeutic benefits.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this work.

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