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Review of *Sangre de Drago* (*Croton lechleri*)—A South American Tree Sap in the Treatment of Diarrhea, Inflammation, Insect Bites, Viral Infections, and Wounds: Traditional Uses to Clinical Research

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ABSTRACT

Objective: The objective of this review is to provide an overview of the pharmacologic evidence that may or may not support clinical and ethnomedical uses of the sap of *sangre de drago* (dragon's blood; *Croton lechleri* Müll. Arg.). Data sources used were BIOSIS, EMBASE, PubMed, TOXLIT, International Pharmaceutical Abstracts, manual searches, papers on file from peer-reviewed journals, textbooks available at Armana Research, Inc., and researchers in the field of South American botanical medicine.

Conclusions: The results of *in vitro* and *in vivo* studies largely support the majority of ethnomedical uses of *sangre de drago* including the treatment of diarrhea, wounds, tumors, stomach ulcers, herpes infection, the itching, pain and swelling of insect bites, and other conditions. Clinical studies of *sangre de drago* products have reported positive results in the treatment of traveler's and watery diarrhea and the symptoms of insect bites. Because the sap has shown low toxicity and preparations used in clinical studies were well tolerated, further clinical and pharmacologic studies are anticipated. Acknowledgment of the diversity in the chemical makeup of the sap from one geographic area to another and the recent characterization of alkaloid chemotypes of *sangre de drago* will require that materials developed for clinical use are standardized.

BOTANICAL DATA

Classification and nomenclature

The scientific name of *sangre de drago* (dragon's blood) is *Croton lechleri* Müll. Arg. (syn. *C. draconoides* Müll. Arg.). Closely related South American species known as *sangre de drago* include *Croton palanostigma* Klotzsch (syn. *C. benthamianus* Müll.-Arg.) (Brako and Zarucchi, 1993), and *C. erythrochilus* Müll.-Arg., both found in Peru (Pieters et al., 1990). Some Peruvian botanists classify *C. draconoides* (Müll. Arg.) as synonymous with *C. palanostigma* (Klotzschs) (Pieters et al., 1990). In central Peru (Oxapampa, Pasco), two other species known locally as *sangre de drago* are *C. perspe-*

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ciosus Croizat and the recently reported *C. rimbachii* Croizat (Meza, 1999). *C. urucurana* Baillon occurs in southeast Brazil (Peres Marize et al., 1997), Paraguay (Portillo et al., 2001), and in Brazil. In Brazil, the herb is also known as *sangre de drago* or *Sangra d'Água* (Lopes Pereira Peres et al., 1998). Brazilian populations of this species are in rapid decline (Peres Marize et al., 1997).

From the family Euphorbiaceae, some common names include *Croton lechleri* (dragon's blood, [English]), *Sangre de Dragón* (Soukup, 1970), *sangre de drago* (Duke and Vasquez, 1994), *Sangre de Grado* (blood of the tree) (Milanowski et al., 2002), and *Palo de Grado* (tree of gladness; Peruvian mestizo) (Peres Marize et al., 1997). The American Herbal Products Association assigns the common names "dragon's blood croton" and *Sangre-de-Drago* as acceptable names for use in U.S. commerce (McGuffin et al., 2000). Aboriginal names for *Croton* species that produce a red latex are far more numerous. In eastern Ecuador, Quechua names are *arleiia* and *lan huiqui*.* In Ayacucho, Peru, the Quechua name is *yawar gradwascca*. Among the Asháninka, the largest Indian tribe in Peru, the tree is known by the names *irariki*, *irari*, and *quirari*, depending on the geographical area of the tribe. In Peru there are at least 20 other indigenous names for *sangre de drago* trees, some translating to wood's blood or tree's blood (Meza and Pariona, 1999).

Dragon's blood is a name of Old World extraction, earlier applied to the plant proper or the sap derived from *Dracaena draco* L. (Liliaceae) of the Canary Islands (a palm tree that produces a red resin used in varnishes), and to the Arabian *D. cinnabari* Balf., the red resin of which was also used in varnishes and in medicine to stop hemorrhages. Other plants known as dragon's blood include the rattan palm of Malaya, *Daemonorops draco* Blume (Palmaceae), which supplied a resin used in photoengraving, etching and in the varnishes of Italian violins during the eighteenth century; and in Guyana, *Pterocarpus draco* L. (Leguminosaceae) or *padauk* supplied West Indian Dragon's Blood (Emboden, 1974; Uphof, 1968).

Description

C. lechleri is described as a medium-sized tree that occurs in forests and disturbed areas from sea level to 1000 meters in the eastern lowlands of the Peruvian Amazon and low mountainous areas of the Peruvian Andean region, as well as Colombia, Bolivia, and Ecuador (Brako and Zarucchi, 1993). In northwest Amazonia, *C. lechleri* is most commonly found at elevations of 100–600 meters. In Ecuador, *C. lechleri* occurs in the primary rain forest as an understory tree where it reaches a height of 15 meters. Much like alder trees (*Alnus* spp.) in the northern hemisphere, *C. lechleri* is a fast-growing (10–15 meters in 3 years) (Miller et al., 2001) pioneer species and is one of the first plants to appear in recently cleared areas and along roadsides (Ubillas et al., 1994). It has heart-shaped (cordate), alternate leaves that range in size from 15–30 cm in width and length and appear with 6–8 parallel veins diverging diagonally from the leaf midvein. The fruits are three-celled and the small flowers appear on a tall thin spike that measures 30–50 cm in length. When cut, the trunk produces a bright red latex that may appear orangeish (Castner et al., 1998).

The yield of latex from *C. lechleri* is greatest in the rainy season and also depends on the age of the tree. The traditional method of slashing the bark produces a maximum yield of several liters of sap from a tree up to 6 years old with a diameter of, on average, 25 cm at breast height. Felling the tree and scoring the bark produces 5–6 L of sap. Because the sap is slowly released from a standing tree, felling and scoring is the preferred method for industrial scale production (Ubillas et al., 1994).

HISTORY AND TRADITIONAL USES

The main part of *C. lechleri* used medicinally in South America is the blood-red latex or sap, which is a common household remedy used in Peru, other Latin American countries, and among the Latin American population of the United States. Although its medicinal uses are still largely unrecognized outside of Latin

*Marles R. The Ethnopharmacology of the Lowland Quichua of Eastern Ecuador [dissertation]. Chicago: University of Illinois at Chicago, 1988.

America, *sangre de drago* recently became available in the United States as a dietary supplement.

The sap of *C. lechleri* is widely sold in the local markets of Ecuador and Peru where it is popularly used to treat diverse illnesses in adults, children, and infants. Internal ethnomedical uses include the treatment of diarrhea, dysentery, cholera (Carlson and King 2000), coughs, flu, lung problems, stomach ulcers, (Ubillas et al., 1994), and hives, the latter being treated by taking the sap in pineapple juice (20 drops per 200 mL). In the upper Amazon, the sap is taken diluted in hot water to speed internal healing after an abortion, and used as a vaginal douche after childbirth (Castner et al., 1998). Others report that the sap is used in Amazonia in vaginal baths taken before childbirth (Duke and Vasquez, 1994) and another refers to the danger of irritating tissues by applying *sangre de drago* after childbirth (Soukup, 1970). These uses probably followed the so-called doctrine of signatures; however, it is noted that *Croton* species are extensively used all over the world for pain- and blood-related health problems, and especially those associated with menstruation.*

In upper Amazonia, the sap is taken to treat tuberculosis and bone cancer (Castner et al., 1998) and may be combined with other medicinal plants to treat other types of cancer (e.g., *Uña de Gato* or *Uncaria tomentosa* [Willd.) DC.]) (Maxwell, 1990). The sap of a closely related species, also known in Peru as *sangre de drago* (Duke and Vasquez, 1994) (*Croton palanostigma* Klotsch), has been popularly used by indigenous people of the region of Pucallpa, Peru, to treat tumors (Hartwell, 1969; Rutter, 1990).

External use of the sap to stop bleeding of cuts and wounds led to the common rural name in Peru of "liquid bandage." While in Peru I learned that it was not unusual to find the sap in the household medicine cabinet next to the iodine, which has a similar appearance. From what I could tell, it was just as common an item in the cities as in the countryside. In the suburbs of Lima, I witnessed the application of the sap to a 6.35-cm wound on the inside arch of the foot of a young man who incurred the injury while surfing nearby. He said that the fin of the board had cut his foot during a fall. His mother applied a few drops of the sap and applied a small bandage. Six hours later at a nightclub in the city, I found him dancing. When I enquired about the wound he claimed to not have any pain (Jones, 1995). On further questioning, he claimed to have taken nothing that would relieve pain except for the distraction of the music and a couple of beers.

Other external ethnomedical uses of the sap in Peru and Ecuador include the topical treatment of bites and particularly stings, for which the sap is said to stop itching and pain in a matter of minutes and to subsequently reduce the attendant redness and swelling (Miller et al., 2000).

The sap is also used in the healing of open sores (oral and otherwise), herpes infections, surgical operations (urban areas) (Ubillas et al., 1994), and infected gums. The Quijos Quichua of eastern Ecuador soak a piece of cotton with the sap, which is applied to alleviate the pain of tooth extractions and cavities.* *C. palanostigma*, the closely related species noted above, is also used to treat pain. In the region of Manaus, Brazil, the sap of this tree is used topically in the treatment of painful boils and ulcers (Schultes and Raffauf, 1990). Still other ethnomedical uses of the sap of *C. lechleri* in Peru are found in the treatment of bone fractures, leucorrhea, piles, hemorrhoids (Soukup, 1970), and rheumatism (Persinos Perdue et al., 1979; Phillipson, 1995).

C. lechleri is a fast-growing pioneer species (Miller et al., 2001; Ubillas et al., 1994), growing as much as 3–4.5 meters per year. Because repeated tapping of the sap renders the trees susceptible to fungal infections, one of the current practices of commercial harvest involves a 2–3-year cycle of felling the trees, draining them of sap, and replanting (Miller et al., 2001). The practice of replanting requires careful management and conservation in conjunction with the indigenous peoples who reside in the forests where they grow (Ubillas et al., 1994). In the course of sustainable management studies of *sangre de drago* in Ecuador and Peru, it

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was learned that unlike the related rubber tree (*Hevea* spp; family Euphorbiaceae), the compartments in the bark of *Croton* species that produce the latex (lactifers) are nonregenerating, which explains why the latex of standing trees does not flow continuously after tapping (King et al., 1997; Ubillas et al., 1994; Castro and Meza, 1999). These studies also found that 10 months after tapping the bark of standing trees to obtain the latex (300 mL), there was a high rate of mortality (44%) or impending death (35%). Only a few trees (2.5%) showed the appearance of surviving. Similar results were seen in populations from different locales in which the amount of sap obtained ranged from 200 to 300 mL (King et al., 1997).

CHEMISTRY

Alkaloids

The leaves of *C. lechleri* contain the morphinandienone alkaloid, sinoacutine (Carlin et al., 1996). The sap contains the phenanthrene alkaloid taspine (Persinos Perdue et al., 1979), which is also found in the sap (Itokwa et al., 1991) and leaves of *C. palanostigma* (Bettolo and Scarpati, 1989). Magnoflorine, isoboldine, norisoboldine, taspine (Milanowski et al., 2002), glaucine, and thaliporphine were identified in the leaves (Bettolo and Scarpati, 1989). The content of taspine in the sap varies widely. A recent survey in northwest Peru and central Ecuador of 493 trees in 20 sites (February/March 1996, March/April, 1999 and September 1997), along with multiple samplings at 13 of the sites indicates that the content of taspine in the latex of *C. lechleri* ranges from 1.3%–20.4% with an approximate mean level of 9% (dry weight), but that it is only found in mature trees. Samplings from a few trees calculated to be a year old contained other alkaloids. Based on analyses of leaves from 264 trees, the survey also found evidence to suggest that there may be three alkaloid chemotypes of *C. lechleri*. The leaves of chemotype 1 contained glaucine, isoboldine, and thaliporphine. Those of chemotype 2 contained isoboldine and thaliporphine while the leaves of chemotype 3 contained only isoboldine. Yet both the content of taspine and the alkaloid profile of the latex of mature trees of the three proposed chemotypes showed no significant difference (Milanowski et al., 2002).

Phenolic compounds

Lignans. Dihydrobenofuran lignan (3′,4-*O*-dimethylcedrusin) and a coniferyl alcohol previously found in species of pine (*Pinus*) were isolated from the sap of *C. erythrochilus* (Pieters et al., 1990).

Tannins. The major constituents of the sap of *C. lechleri* are proanthocyanidins and flavonols (Cai et al., 1991). The sap is abundant in soluble proanthocyanidins (also referred to as procyanidins, condensed tannins or procyanidin oligomers or PCOs), containing up to 90% by dry weight (Cai et al., 1991). Upon heating in acid medium, PCOs yield cyanidin. A mixture of PCOs known as SP-303 (molecular weight ~2100 da) isolated from the sap of *C. lechleri* is largely composed of (−)-galloepicatechin and (+)-gallocatechin with lesser amounts of (−)-epicatechin and (+)-catechin (Ubillas et al., 1994). SP-303 has the appearance of a dark reddish-brown powder (Ubillas et al., 1994). Other related compounds found in the sap of *C. lechleri* (Ecuador) are procyanidins B1 and B4 (Cai et al., 1991).

Terpenoid compounds

Diterpenes. Diterpenes isolated from the bark of *C. lechleri* (Ecuador) were found in minor amounts in the sap: bincatriol, crolechinol, crolechinic acid, hardwickiic acid, and koberins A and B (Cai et al., 1993).

Steroids. The bark of *C. lechleri* (Ecuador) contains β-sitosterol-β-D-glucopyranoside and β-sitosterol (Cai et al., 1993).

To date, procyanidins and alkaloids are considered to be the most active constituents of the sap.

PRECLINICAL STUDIES

Gastrointestinal functions

Antisecretory activity against diarrhea. A survey of local ethnomedical uses of the latex in the area of Iquitos, Peru, in 1996 by ethnob-

otanist Franklin Ayala and Peruvian registered nurse Dina Ayala, found that 57% of the randomly interviewed populace reported its use in the treatment of diarrhea (Carlson et al., 2000). While this application was already known (Ubillas et al., 1994),* no one had reported any frequency of use. The fact that *sangre de drago* was also taken orally in Peru to treat watery diarrhea (dysentery and cholera) encouraged researchers to initiate studies on its potential use against this illness (Carlson et al., 2000).

Using a mixture of procyanidin (proanthocyanidin) oligomers derived from the latex and designated SP-303, researchers examined antidiarrheal activity in a mouse model for secretory diarrhea with cholera toxin as the inducer. In the form of enteric-coated beads, SP-303 (100 mg/kg by gavage) administered at the same time as the cholera toxin caused a significant and dose-dependent reduction in the amount of toxin-induced fluid accumulation in the small intestine. Levels of fluid were nearly restored to normal. Administered 3 hours after mice were treated with the cholera toxin, SP-303 again produced a dose-dependent inhibition of fluid accumulation. At the highest dose tested (50 mg/kg), fluid accumulation levels were not significantly different from those of controls. The half-maximal inhibitory amount of SP-303 against cholera toxin-induced fluid accumulation was approximately 10 mg/kg (Gabriel et al., 1999).

In vitro studies to determine the mechanism involved were performed by elevating cyclic adenosine monophosphate (cAMP) levels in intestinal epithelial cells using a potent activator of adenylate cyclase (forskolin, derived from *Coleus forskohlii* Briq. syn. *C. barbatus* Benth.), thereby modeling the effects of cholera enterotoxin. Binding of the enterotoxin to intestinal cells leads to modification of a stimulatory protein (G protein) and subsequent activation of adenylate cyclase which in turn elevates cAMP. Consequently, a chloride channel is activated which causes a high volume of chloride and resultant fluid secretion in the intestine. Left untreated this state can result in dehydration and death. SP-303 was shown to inhibit cAMP-mediated chloride secretion in intestinal epithelial cells (Caco-2 cells and T84 cells). Maximal inhibition of forskolin-stimulated chloride secretion in the intestinal cells was found from the addition of 300 μM of SP-303. The concentration that inhibited the chloride secretion by 50% (IC_{50}) was approximately 50 μM (Gabriel et al., 1999).

Further *in vitro* studies on the mechanism of the antidiarrheal activity of *sangre de drago* were conducted using a mixture of the whole sap collected from *C. lechleri* and *C. palanostigma* (Upper Huallaga River Valley, Amazonian Peru). Pretreatment of isolated guinea pig ileum with the sap (1:1,000) inhibited chloride secretion evoked by capsaicin (derived from chili peppers, *Capsicum annuum* L.) by approximately 70%. Because the response to capsaicin is mediated by substance P released from sensory afferents (inner part of nerves), these results suggest that the sap suppressed epithelial secretion by some direct inhibitory effect on sensory afferent activation. The results of *in vitro* tests indicated that the sap mixture does not compromise cholinergic, substance-P–dependent epithelial (neuron-induced) secretion, indicating that the sap does not act as a general nerve activation-inhibiting substance. The researchers postulated that because of its ability to block sensory afferents activated by capsaicin, the sap may attenuate the pain and cramping that attends the secretory processes of diarrhea as well as intestinal distress. They add that it may also have therapeutic use in other types of neurogenic inflammation (Miller et al., 2000).

Recent evidence indicates that fluid secretion caused by rotavirus involves enteric nervous system activation in the wall of the intestines (Lundgren et al., 2000). Therefore, the ability of the sap to block capsaicin-activated sensory afferents (Miller et al., 2000) may represent a possible or new means of treating rotavirus diarrhea, which may become an important use of the sap. Rotavirus is the major cause of severe diarrhea in young children and infants worldwide and by the age of 5, almost every child will experience rotavirus gastroenteritis. The virus causes dehydration, nausea, vomiting,

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and diarrhea, and causes an estimated 352,000–592,000 (median, 440,000) deaths worldwide per year largely in the developing world (Parashar et al., 2003; World Health Organization, 1999). Worldwide, rotavirus causes approximately 2 million hospitalizations, 25 million visits to a clinic, and 111 million episodes of gastroenteritis every year (Parashar et al., 2003). Since the discovery of rotavirus in the 1970s, a vaccine was developed and then withdrawn in 1998 after producing undesirable side-effects (Dennehy and Bresee, 2001). Since then, the need for effective and affordable treatment has become more urgent (Parashar et al., 2003). In the United States among children 5 years of age and younger, the exact incidence of rotavirus-associated diarrhea has yet to be determined with any certainty. However, recent trends in the United States incidence of diarrhea in this age group suggest that it may be more prevalent than previously thought. Beginning a year after rotavirus infection was specifically coded for in U.S. hospitals in 1993 to 3 years later, among insured patients in the age group the incidence increased from 6.9% to 17.7% of all diarrhea-associated hospitalizations, resulting in 593 children being hospitalized and more than 6000 outpatient visits (Zimmernan et al., 2001).

Immune functions: inflammation and disease

Cytotoxicity against cancer cells. Potent *in vitro* cytotoxicity against KB cells (human oral epidermoid carcinoma) was reported from the alkaloid taspine. The concentration required to inhibit KB tumor cell growth by 50% (IC_{50}) was 0.39 μg/mL (Itokwa et al., 1991). Further tests against the growth of KB cells were conducted using the sap from *C. lechleri* collected in Ecuador. At greater than 900 μg/mL (Chen et al., 1994), the IC_{50} of the raw sap was much higher than that obtained in an earlier study on KB cells with sap from Peruvian *C. palanostigma* (Itokwa et al., 1991), a result likely caused by the trace amount of taspine in the sap from Ecuador (Chen et al., 1994) versus a taspine content of at least 10 mg/g in the Peruvian sap (Itokwa et al., 1991). After freeze-drying the sap from Ecuador (*C. lechleri*), the IC_{50} in the KB cell assay was approximately 4.8 times smaller (IC_{50} 187 μg/mL). However, even this concentration was hardly cytotoxic and various solvent extracts of the sap failed to show much higher activity. A methanolic extract of the heartwood was more active (IC_{50} 25 μg/mL), but was still not cytotoxic. With an IC_{50} of more 20 μg/mL, various constituents isolated from the sap also showed no cytotoxic activity. The most active compound was 1,3,5-trimethoxybenzene (IC_{50} of 7.13 μg/mL) which was still much weaker than the control (emetine hydrochloride, IC_{50} of 0.2 μg/mL). It was proposed that if any *sangre de drago* sap with a low content of taspine can produce antitumor activity, it may be because of mechanisms other than cytotoxicity, such as immunostimulation (Chen et al., 1994).

More recent *in vitro* studies on the tumor-cell cytotoxicity of *sangre de drago* examined effects on human erythroleukemia K562 cells (Rossi et al., 2003) and human gastrointestinal cancer cell lines of colon (T84 and HT29) and stomach cancer (AGS) (Sandoval et al., 2002). Dose-dependent antiproliferative activity against K562 cells was found from reconstituted, filtered, freeze-dried raw sap of Ecuadorian *C. lechleri* (IC_{50} of 2.5 μg/mL) collected in Morona Santiago province (Rossi et al., 2003). In the study on colon and stomach cancer cell lines, the sap of *C. palanostigma* (Upper Huallaga Valley, Tingo Maria, Peru) was also reconstituted in water from filtered, freeze-dried material and used in all the experiments. After incubation with the sap at a concentration of 100 μg/mL and 200 μg/mL, but not at 10 μg/mL, cell viability, cell adhesion, and cell proliferation were significantly decreased in all the cancer cell lines. Cell adhesion of the cancer cells was shown to be irreversibly damaged with complete loss of adhesion when the cells were taken to another medium. Apoptosis was significantly increased at the same concentrations of the sap in each of the cancer cell lines and in each experiment the effect of the sap was concentration and time-dependent. Effects of the sap (100 μg/mL) on microtubule morphology were similar in each of the cancer cell lines. Exposure to the sap caused clumps to form and the microtubule structure to undergo significant damage, although more so in the T84 colon cancer cells. Coupled with the observed effects

of the sap on microtubule structure and damaged adhesion ability of the cancer cell lines, Sandoval et al. (2002) concluded that it can induce changes similar to those of the anticancer agent Taxol™ (Bristol-Myers Squibb, Princeton, NJ), which also renders microtubules nonfunctional and results in cellular apoptosis and the inability of cancer cells to adhere. As to what the active constituents may be, the researchers suggested the possibility of vanilloid compounds (Sandoval et al., 2002).

Antimicrobial activity against infectious diseases. The freeze-dried sap of *C. lechleri* (Ecuador) showed weak activity against the growth of *Bacillus subtilis* (strain JTS 13) and *Escherichia coli* (strain KL 16), with activity only at concentrations of greater than 10 μg. A methanolic extract was not much better and ethyl alcohol and acetone extracts were less active than the freeze-dried sap. Better activity against *E. coli* was found from a chloroform extract, but it was less active against *B. subtilis*. Among various constituents of the sap, highly potent activity against *B. subtilis* was found from two compounds (2,4,6-trimethoxyphenol and 1,3,5-trimethoxybenzene at 0.0003 μg) which showed 30-fold the activity of chloramphenicol and penicillin. These substances were also highly active against the growth of *E. coli*, although less potently so (1.0 and 0.04 μg, respectively). High activity was also found from several diterpenoid constituents of the sap. Crolechinic acid was active against *E. coli* and *B. subtilis* (1.0 and 0.2 μg, respectively), and at 0.04 and 0.05 μg, korberins A and B showed good activity against *B. subtilis* (Chen et al., 1994).

A combination of the undiluted saps of Peruvian *sangre de drago* (*C. lechleri* and *C. palanostigma*) showed 100% lethality against *E. coli* and was still 90% lethal at a dilution of 1:10 (Miller et al., 2000).

Antiviral activity against viruses. Antiviral activity of the procyanidin preparation known as SP-303 (molecular weight approximately 2100 da) has been more extensively tested than any other constituent of the sap (Ubillas et al., 1994). SP-303 has shown *in vitro* activity against *Herpes simplex* viruses (HSV-1 and HSV-2), inhibition of thymidine kinase mutants of the viruses, and pronounced activity against acyclovir-resistant strains (Barnard et al., 1993; Safrin et al., 1993). In the plaque reduction assay, SP-303 exhibited greatest potency against various isolates of HSV-2 (ED_{50} of 0.9–2.1 μg/mL). Evidence of SP-303-induced interferon production was absent (Barnard et al., 1993) and its function was not like that of ribavirin, which inhibits viruses during the replication stage. Studies suggest that the mechanism of viral inhibition of SP-303 is at the level of plasma membrane penetration and/or adsorption at an early stage of viral activity (Barnard et al., 1993; Ubillas et al., 1994).

At 6 hours postinfection, guinea pigs vaginally infected with HSV-2 showed significantly less viral lesions after topical treatment with a dimethyl sulfoxide (DMSO; 78% w/w)-based ointment containing SP-303 (10%). The ointment was approximately half as active as acyclovir (5% ointment). Similar results were found in mice vaginally infected with HSV-2 after treatment with a 10% SP-303 cream or SP-303 administered orally (90 mg/kg per day for 8 days) (Ubillas et al., 1994). The mean lesion score of mice topically treated with SP-303 (10%) was significantly reduced and 70% of the animals survived (versus 100% of those treated with acyclovir). Benefits from intraperitoneal (30 mg/kg per day) and oral SP-303 (270 mg/kg twice daily) were not significantly different from the 10% topical cream. In uninfected control mice, no signs of irritation were found from the topical preparation; however, intraperitoneal (i.p.) and oral doses of SP-303 caused weight loss (Barnard et al., 1993).

Administration of SP-303 (9 mg/kg per day) by small particle aerosol to mice infected with influenza A produced significant increases in survival and significantly decreased pulmonary influenza titers, damage to lung tissues, and development of pneumonitis. However, neither oral or i.p. administrations of SP-303 produced statistically significant results (Gilbert et al., 1993).

Respiratory syncytial (RSV) and parainfluenza viruses are leading causes of serious infections of the lower respiratory tract of children less than 2 years old. In infants, RSV can cause pneumonia and bronchiolitis. It also

causes acute respiratory infections in the elderly. In young children and infants, parainfluenza viruses cause the common cold as well as otitis media, bronchiolits, severe croup, and pneumonia (Bennett and Plum, 1996).

SP-303 selectively inhibited several respiratory viruses *in vitro* (Wyde et al., 1991) and appeared to inhibit the cellular penetration of RSV (Barnard et al., 1992). Against RSV infection in rats, single doses of SP-303 (1–10 mg/kg per day i.p.) produced significant reductions of 75% to 97% in pulmonary titers of the virus compared to placebo. The highest dose produced results comparable to ribavirin (90 mg/kg i.p., 99% reduction in virus titer) and provide the only consistent results. Oral administration of SP-303 produced variable results against RSV infection. Significant results compared to placebo were seen from twice daily doses of 3 mg/kg orally (80% to 99% reductions in viral titers, $p = 0.03$). However, doses of 1 mg or less and 30 mg or more twice daily failed to produce consistently significant results. Significant reductions in titers of parainfluenza virus type 3 (PIV3) of 87% to 94% were found in rats treated with SP-303 at single doses of 3 mg/kg and 10 mg/kg per day i.p. respectively, compared to placebo (Wyde et al., 1993).

Oral dosages of SP-303 (30, 90, or 270 mg twice daily 4 hours prior to infection for 7 days) produced significant decreases of RSV titers in African green monkeys infected by inoculation (Soike et al., 1992). Administered 24 hours after infection, oral dosages of SP-303 at 10 or 90 mg/kg per day produced significant reductions in viral titers of the lungs (Ubillas et al., 1994). No generalized toxic effects or changes in clinical chemistry were found from oral doses of 100, 300, or 900 mg/kg per day for 5 days (Soike et al., 1992).

In cultures of several tumor viruses (simian sarcoma virus type I, Rauscher murine leukemia virus, and avian myeloblastosis virus), the alkaloid taspine (70–98 μg/mL) inhibited the enzyme reverse transcriptase by 50% (Sethi, 1977).

Inflammatory response

Arthritis. In an animal model of polyarthritis, the anti-inflammatory activity of the alkaloid taspine was compared to that of indomethicin (1 mg/kg per day orally). Male rats administered the alkaloid (20 mg/kg per day orally) for 3 days prior to adjuvant-induced arthritis and for 17 days thereafter showed a significant decrease in paw swelling, which was comparable to or greater than that of indomethicin. In a separate study on edema in rats (carrageenan-induced pedal edema), taspine (median effect dose [ED_{50}] 58 mg/kg orally) displayed 3–4 times the anti-inflammatory potency of phenylbutazone (Persinos Perdue et al., 1979).

Gastric ulcers. In a rat model of gastric ulcer (acetic acid-induced), sap derived from Peruvian *C. lechleri* and *C. palanostigma* (Rainforest Phytoceuticals, Delmar, NY) administered in drinking water for 7 days (60 or 600 μg/ = 1:10,000 or 1:1000 dilution) produced a significant reduction in the size of ulcers. In contrast to untreated rats, the gastric epithelium of the sap-treated rats showed areas of regenerating epithelia. At either concentration, the magnitude of the healing from the sap was at least as great as that from a combination of streptomycin and penicillin. Moreover, tests revealed a significant decrease in bacterial counts of the ulcers in the sap-drinking rats versus controls. Subsequently, the undiluted combination of saps was found to kill *E. coli* completely. Even at a dilution of 1:10 the sap combination was still 90% effective. A further benefit from the sap was seen in a significant decrease in the granulocyte contents of the ulcers, which was evident in greatly decreased ulcer myeloperoxidase levels. The researchers noted that this effect was something not found in previous studies of ulcer-healing using probiotics or antibiotics (Miller et al., 2000).

In the gastric epithelium of rats with gastric ulcers, Miller et al. (2000) found highly upregulated gene expression of the cytokines (messengers) tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS), interleukin-6 (IL-6), interleukin-1β (IL-1β), and cyclo-oxygenase-2 (COX-2). In the gastric epithelium of the sap-treated rats, gene expression of the proinflammatory cytokines was reduced, especially iNOS gene expression (Miller et al., 2000). In studies on chronic ileitis (Miller et al.,

1993), chronic granulomatous colitis (Grisham et al., 1994), and *Heliobacter pylori* gastritis (Mannick et al., 1996), the expression of iNOS has been associated with locations of tissue injury. Therefore, the ability of the sap to reduce iNOS suggests that it may hold promise against these conditions as well.

Neurogenic inflammation. Miller and colleagues (2001) hypothesized that the ethnomedical uses of *sangre de drago* as a wound-healing, analgesic, and antidiarrheal agent, and its purported ability to relieve pain and itching quickly, might derive from a suppressive effect on sensory afferent nerve activation. Sensory afferent nerves are found in the skin, lungs and gut where they act as sentinels for nerves, transmitting impulses from the periphery to the central nervous system. Further support for the hypothesis came from one of the authors who found the burning sensation caused by exposure of his mucosal skin to capsaicin was relieved by applying the sap (Miller et al., 2001). The results from an ensuing series of experiments support their hypothesis.

Increased sensitivity to pain (hyperalgesia) induced in the paws of rats by intradermal protease activated receptor-2 activating peptide (PAR-$_2$AP) was abolished by a single topical pretreatment of the sap in the form of a balm containing 1% *sangre de drago* (Zangrado Bug Bite Balm, Rainforest Phytoceuticals, LLC). Because this effect was absent in rats not treated with PAR-$_2$AP, an anesthetic action of the balm was not indicated. Hyperalgesia induced by intradermal prostaglandin E_2 (PGE_2) was completely blocked in rats treated with the balm in a single topical pretreatment. A single topical application of the balm to the paws of rats with PAR-$_2$AP-induced edema caused the volume of swelling to reduce by approximately 50% compared to placebo ($p < 0.01$). The effect lasted 6 hours or more and the reduction in edema was more sustained and quicker than the natural decline evident in the placebo group. In another experiment, phenylephrine-constricted rat mesenteric arteries relaxed in response to the addition of calcitonin gene-related peptide (CGRP), one of the main neurotransmitters of sensory afferent nerves. Upon introduction of the sap in dilution (1:10,000), the response to CGRP was significantly inhibited ($p < 0.05$), indicating that the sap inhibited the CGRP receptor. An active constituent in the sap (molecular weight 930) that appeared to be a procyanidin oligomer was found to be just as active as the sap itself against PAR-$_2$AP-induced hyperalgesia. In addition, compared to vehicle-treated controls, excessive mucosal blood flow in the stomach of rats (gastric hyperemia or gastric mucosal vasodilation) induced by capsaicin was largely prevented by pretreatment with 1% solutions of the sap or the active constituent (each $p < 0.01$). Taken together, these results indicate that the sap directly blocks sensory afferent nerve activation at both the prejunctional and postjunctional level; this is a dual action, which the researchers claim, appears to be unique to *sangre de drago* (Miller et al., 2001).

Connective tissue functions

Wound repair. The traditional use of *sangre de drago* as a liquid bandage in the Amazon led several research groups to investigate alleged wound-healing activity. Although no one constituent can be singled out as responsible, early investigations attempted to do just that. Chen and colleagues (1994) emphasized that what became obvious in their work with the sap of Ecuadorian *C. lechleri* was considerable differences in chemical composition of *sangre de drago* saps from different origins. They further concluded that the wound-healing properties of the sap may be the result of various factors: The ability to form a film that protects against microbial invasion of wounds; free radical scavenging activity of procyanidins; the high content of polyphenolics with their well-known aspect of binding proteins and enzymes; and the anti-inflammatory and strong antibacterial action of polyphenols, which together would facilitate improved healing of damaged tissue (Chen et al., 1994).

Vaisberg et al. (1989) reported that twice-daily topical application of 0.05 mL of a 10% solution of the sap (*C. lechleri*) to skin wounds of mice caused a significant 31% increase ($p < 0.05$) in the rate of wound repair. Taspine was found in the sap at a concentration of 0.1–0.2 mg/mL and through a bioassay for cicatrizant

activity was isolated as the active constituent. Topical application of 0.05 mL of a 10% solution of taspine hydrochloride every 12 hours also produced a significant wound-healing activity of 58.2% ($p < 0.005$). The effect was dose-dependent with concentrations of up to 13.2 μg per mouse producing increasing rates of wound healing (cicatrizant) activity. A higher dose (40 μg per mouse) produced only a 23.1% increase in wound-healing activity, indicating a threshold at which the activity may be optimum from taspine. In an *in vitro* test, wounding of human fibroblasts was performed in order to count the population of cells that migrate to the repair the area of damage. Fibroblasts treated with taspine showed a significant increase in numbers of migrating cells, suggesting that this effect may be at work in the wound-healing activity of the sap and of taspine (Vaisberg et al., 1989).

Pieters and colleagues (1992) also reported that the sap was active as a wound-healing agent. Evidence was obtained using an ethyl alcohol extract in an assay for stimulation of human endothelial cells (umbiblical vein). The use of this assay is predicated upon the role of endothelial cells in the healing process of skin tissue; when new tissue forms, endothelial cells proliferate to allow the formation of new blood vessels. Cellular proliferation was measured according to the rate that radio-labeled thymidine (^3H-thymidine) incorporated into cellular DNA in the presence of the test substances. From the sap of Peruvian *sangre de drago* (*Croton* sp.), they isolated an active constituent; a lignan known as 3′,4-*O*-dimethylcedrusin (4-*O*-methyldihydrodehydrodiconiferyl alcohol). The concentration of the lignan in the sap (approximately 14 μg/mL or 0.0014%) was the same as that which produced positive activity in the wound-healing assay. The lignan was proposed to be the active constituent because it also protected endothelial cells from undergoing degradation in a starvation medium and it stimulated endothelial cells (Pieters et al., 1992). However, at higher concentrations (125 and 250 mg/mL) incorporation of radiolabeled thymidine into the cells was inhibited by the lignan, as well as by taspine (0.5 μg/mL and more), which was otherwise inactive in the assay (Pieters et al., 1993).

Porras-Reyes and colleagues (1993) focused on the wound-healing activity of taspine, which became "the first plant alkaloid confirmed to accelerate wound healing" (Porras-Reyes et al., 1993). DMSO allowed taspine to be diluted and served as the vehicle control treatment for *in vitro* and *in vivo* studies. Taspine was otherwise highly insoluble. In a linear incision model in rats, a single topical application of taspine (250 μg dissolved in 0.1 mL of DMSO) produced a significant increase in the tensile strength of wounds. At 5 and 7 days postincision, wound tensile strength showed significant increases of 26% and 30% compared to vehicle-treated control incisions ($p < 0.005$ and $p < 0.0001$, respectively). By day 12, however, there was no difference in wound strength. Smaller doses of taspine (10 and 50 μg/mL) failed to produce any increases in wound tensile strength. A number of tests were performed to determine how taspine accelerated wound healing. On days 5 and 7, the influx of mononuclear cells in the taspine-treated (250 μg/mL) wounds was higher compared to controls. Fibroblast infiltration was not stimulated by taspine. Rather, cell viability was decreased and thymidine incorporation was inhibited (Porras-Reyes et al., 1993). The negative effect was probably the result of *in vitro* toxicity, as previously reported by Vasiberg and colleagues (1989). A positive effect on the expression of fibronectin was suspected as a possible means of accelerated wound healing by taspine, but *in vitro* tests showed that fibronectin matrix disposition was not affected. The chemotactic properties of macrophages were likewise not affected, although fibroblast chemotactic activity was; optimal promotion of fibroblast migration from taspine was seen at 50 pg/mL. In conclusion, Porras-Reyes et al. (1993) thought increased fibroblast migration to be the likely means by which taspine enhanced wound healing.

Chen and coworkers (1994) examined the wound-healing activity of the sap from Ecuadorian *C. lechleri*, which contained only traces of taspine (Bettolo and Scarpati, 1989) and no 3′4-methylcedrusin (Phillipson, 1995). The assay for activity was the proliferation of endothelial cells (bovine). They also measured thymidine (^3H-thymidine) incorporation to de-

termine the rate of endothelial cell proliferation, as in the previous study. Rather than any increase in endothelial cell proliferation, the dried sap (20 μg/mL) inhibited proliferation/thymidine incorporation by as much as 44%. (Whether drying the sap affected the activity is not known.) Ethyl alcohol, chloroform, and methanol extracts of the sap also inhibited endothelial cell proliferation, whereas an acetone extract was inactive either way. Testing individual constituents of the sap, they found endothelial cell proliferation increased by procyanidin B-4 (10 μg/mL) and most potently by (−)-epigallocatechin and (+)-gallocatechin (each at 5 μg/mL) (Chen et al., 1994). At this, Chen and coworkers pointed out a study by Pieters[†] in which the polyphenolic fraction of the sap, which would contain the aforementioned active constituents, was active in healing the wounds of rats. After treatment with the fraction, wound tissues were reported to have contracted after only one day, "and the wound site was completely covered with a dark crust" (Chen et al., 1994, citing Pieters,[†]). Examined under a microscope 1 month later, Pieters reported that the newly formed tissue was indistinguishable from unwounded tissue. However, Chen et al. (1994) noted that such an effect was not found by Pieters in experiments using 3′4-methylcedrusin or taspine. As for the various constituents of the Ecuadorian sap that contributed to inhibition of endothelial cell proliferation, they found the majority held only slight or no inhibitory activity. The exceptions were korberin A (10 μg/mL, $\geq$ 52% inhibition) and 1,3,5-trimethoxybenzene (0.5 mg/mL, $\geq$ 100% inhibition) that were, respectively, highly and extremely active (Chen et al., 1994). Again, both high and low levels of taspine were shown to be active.

Antioxidant activity

Tests of the antioxidant activity of the sap are lacking. In the total reactive antioxidant potential (TRAP) index, sap collected from Peruvian *C. lechleri* produced results suggesting the presence of antioxidant compounds in high concentration. In an assay to measure effects on free radical-mediated DNA-sugar damage (induced with iron), low concentrations of the sap (1 and 10 μg/mL) increased the level of oxygen radical activity whereas high concentrations (100 μg/mL and 1000 μg/mL) prevented oxidant activity. In contrast, catechin inhibited oxidative DNA damage at a concentration of 1 μg/mL. The sap (1–100 μg/mL) also increased oxidative activity in the hydroperoxide-initiated chemoluminescence assay using rat liver cells. However, in aqueous reaction media the sap prevented oxidative DNA damage and captured hydroxyl and peroxyl radicals, indicating that the constituents responsible are water soluble (Desmarchelier et al., 1997).

Administered to mice by subcutaneous injection, the freeze-dried and redissolved latex of Peruvian *C. lechleri* inhibited hepatic lipid peroxidation as evident in the measurement of malonaldehyde (MDA) thiobarbituric acid reactive substances (TBARS) production in the livers of the animals. However, protection against hepatic lipid peroxidation was only found from a dosage of 200 mg/kg subcutaneously. At 50 mg/kg, MDA levels were found to increase, at 100 mg/kg there was no significant antioxidant activity, and at 300 mg/kg there was evidence of toxicity (Desmarchelier and de Moraes Barros, 2003). Unfortunately, the route of administration is incongruent with traditional uses of the sap. Moreover, a study using the oral route of administration could produce entirely different results (Shanbhag et al., 1990).

Antimutagenic activity

The raw sap collected from Ecuadorian *C. lechleri* prevented the mutagenicity of 2-amino anthracene in both the TA100 (IC_{50} 430 μg/mL) and TA98 (IC_{50} of 340 μg/mL) strains of *Salmonella typhimurium* by 90% and 100%, respectively (Rossi et al., 2003).

CLINICAL STUDIES

Gastrointestinal disorders

Diarrhea. In the United States diarrhea is more often fatal in persons aged 80 and over

[†]Pieters LA. The Biologically Active Constituents of "sangre de drago," a Traditional South American Drug [dissertation]. Antwerp, Belgium: University of Antwerp, 1992.

than in other age groups. Three percent (3%) of all U.S. hospitalizations in 1985 involving diarrhea were comprised of this age group and the rate was far higher than in any other, including children under age 5 (0.05%) (Gangarosa et al., 1992). In the developing world, the incidence of diarrhea in children under age 5 is estimated at 1 billion episodes annually, resulting in an estimated 3.3 million deaths each year (Bern et al., 1992).

As previously noted, *sangre de drago* is frequently used in ethnomedicine for the treatment of diarrhea (Carlson et al., 2000; Marles, 1992; Ubillas et al., 1994). After animal and *in vitro* studies confirming an antisecretory activity of the sap (see Diarrhea section above), placebo-controlled clinical trials of a defined preparation (SP-303) were initiated in traveler's diarrhea, watery diarrhea (Ubillas et al., 1994), and human immunodeficiency virus (HIV)-associated diarrhea (Holodniy et al., 1999). A review of the research on the preparation in the treatment of diarrhea appeared in *HealthNotes Review of Complementary and Integrative Medicine* (Carlson et al., 2000).

Traveler's diarrhea. Traveler's diarrhea is classified as a syndrome comprising an increase in the frequency of unformed stools of 200% or greater (typically, 4–5 loose stools per day) and common symptoms of malaise, fever, nausea, bloating, cramps, and urgency. The episodes often begin abruptly, either while one is traveling or not long after returning home. Although the episodes are in most cases self-limited, rates of attacks range from 20%–50%. Destinations of greatest risk are Latin America, Africa, the Middle East, and Asia (Centers for Disease Control and Prevention, 2001). In at least 80% of cases, traveler's diarrhea is caused by bacterial enteropahthogens (DuPont and Ericsson, 1993) including *E. coli*, *Salmonella*, *Shigella*, and *Campylobacter jejuni* (Centers for Disease Control and Prevention, 2001) and has a tendency to be more severe in Americans traveling to Mexico, for example, than it is in Mexicans traveling in their own country (Carlson et al., 2000). Enterotoxigenic *E. coli* is associated with acute traveler's diarrhea but not significantly with persistent diarrhea (Schultsz, et al., 2000).

The potential of SP-303 as an antidiarrheal agent was evaluated in acute diarrhea in 184 travelers to Jamaica and Mexico. Entry into the trial (double-blinded, randomized, placebo-controlled) was limited to travelers presenting with acute diarrhea who had at least 3 unformed stools in the preceding 24 hours and diarrhea for no more than 48 hours. Subjects were randomly assigned to receive treatment with either placebo or SP-303 at doses of 125 mg, 250 mg, or 500 mg, twice daily for 2 days. Efficacy was determined from 169 subjects who were observed for 24 hours after the 2-day treatment period. No adverse effects were found compared to controls and each dosage of SP-303 was significantly more effective than placebo ($p < 0.05$). Subjects that received 250 mg twice daily showed in more than 90% of cases partial or complete improvement of symptoms in the first 24 hours. Time to the last unformed stool was 38 hours versus 54 hours in the placebo group; a highly significant difference ($p = 0.0002$) (Carlson et al., 2000, citing Dicesare et al., 1998).

Watery diarrhea. A randomized, double-blinded, placebo-controlled trial of SP-303 in the treatment of watery diarrhea was performed in-hospital in residents of Venezuela. Patients were included who presented with diarrhea in moderate and severe acute watery forms. Over a period of 48 hours, SP-303 or placebo was administered in oral doses of either 125 mg, 250 mg, or 500 mg four times per day. Male and female patients ($n = 140$; ages 18–69 years) were enrolled who had experienced at least 5 watery stools in the preceding 24 hours. Time to the last unformed stool was the main endpoint for evaluation of efficacy measured at 24, 48 and 72 hours. The results showed that the treatment was well tolerated, but that only the 125-mg dose was effective and only in reducing the time to the last unformed stool in the 48-hour treatment period versus placebo ($p = 0.02$) (Carlson et al., 2000, citing Ettedgui et al.[‡]). The reason for the difference

[‡]Ettedgui G, Schael IP, Porter S, Pennington J. A double-blind, randomized, placebo-controlled, multi-dose, phase II study to assess the safety and efficacy of SP-303 in the symptomatic treatment of acute diarrhea among adult residents of Venezuela: Oral administration of 125 mg, 250 mg, or 500 mg of SP-303 given every 6 hours for 48 hours. South San Francisco, CA: Shaman Pharmaceuticals, 1998, unpublished.

in the results compared to other studies of SP-303 in diarrhea is not clear. It may have been because of differences in diet, enteropathogens, study design, and/or the fact that these patients were not suffering from traveler's diarrhea.

HIV-associated diarrhea. In up to 90% of cases, patients infected with HIV suffer from diarrhea. The problem grows worse as the immune system becomes more compromised. In the developing world, lack of hygiene, poor sanitation, medications, and even herbal treatments may also cause infections of the gastrointestinal tract. If the cause of the diarrhea can be identified there is some hope of successful treatment, but in up to 60% of cases it remains unknown. In HIV-infected patients the causes may be infectious or noninfectious. Infectious causes include the majority of pathogens affecting people with traveler's diarrhea, plus amebiasis, candidiasis, *Cryptosporidium*, cytomegalovirus, giardiasis, *Isospora belli*, and *Mycobacterium avis* complex. Noninfectious causes of diarrhea in these patients include malabsorption (lactose intolerance, HIV enteropathy, HIV osmotic drink and food), medications (therapy with multiple drugs, traditional herbal treatments), obstruction, incontinence of the rectum, and stress. Patient response to drugs that control motility (e.g., loperamide) has been poor and some just cost too much (e.g., octreotide) (Katabira, 1999).

Clinical trials of SP-303 in the treatment of HIV-associated diarrhea have largely produced positive results (Carlson and King, 2000; Holodniy et al., 1999; Koch et al., 1999; Koch, 2000). A phase II multicenter clinical trial (randomized, double-blinded, placebo-controlled) of SP-303 was conducted by the University of California, San Francisco, in 45 HIV-infected patients diagnosed with diarrhea and acquired immune deficiency syndrome (AIDS)-defining illness or CD4 count less than 200 (males and females ages 18–60 years). Subjects received SP-303 (500 mg orally every 6 hours) or placebo for 4 days. The majority were receiving treatment with antiretroviral agents (80%) and protease inhibitors (77%). All patients stopped treatments for diarrhea 24 hours before enrollment in the trial. For 94% no pathogens were identified in stool samples. Treatment with SP-303 resulted in significant reductions in the frequency of abnormal stools ($p < 0.04$) and of stool weight ($p < 0.008$) compared to placebo, and there were no adverse effects or laboratory abnormalities (Holodniy et al., 1999).

A Phase III multicenter inpatient trial of SP-303 in the treatment of HIV-associated chronic diarrhea (stool weight $>$ 300 g per 24 hours) by the University of California, San Francisco was conducted in 400 patients diagnosed with AIDS. Subjects were men or women 18 years of age or older, the majority of whom were receiving treatment with antiretrovirals and protease inhibitors (93.3% and 83.3%, respectively). Any antidiarrheal agents were discontinued more than 24 hours prior to patient enrollment. After being randomly assigned to either placebo or active treatment groups, subjects received one of three different dosage formulations of SP-303: 250 or 500 mg in a delayed release tablet or 500 mg in the form of delayed release beads (each four times per day for 6 days). Responders to SP-303 were allowed to continue the treatment for another 21 days. The results showed that only those who received the 500-mg tablet benefited. For those with severe diarrhea (stool weights of at least 1000 g per 24 hours), treatment with the 500-mg tablet produced a significant reduction in stool weight ("the primary efficacy endpoint") compared to placebo ($p = 0.008$). No adverse effects were found and laboratory measurements showed no abnormalities (Koch, 2000).

A group of patients ($n = 42$) pooled from the phase III trial and from an open-label study of SP-303 (250-500 mg four times per day) were recruited by Koch and coworkers (1999) for a study on changes in diarrhea-related quality of life (QOL) scores. The researchers pointed out that the influence of antidiarrheal therapy on QOL was previously unknown. The QOL questionnaire included queries on daily living activities, ability to sleep and to perform errands, and of effects on sexual activity. From the results, Koch et al. (1999) concluded that QOL is adversely affected by diarrhea; those who responded to treatment experienced a significant improvement ($p = 0.024$) in the sum score for daily living activities within 2–4 weeks; and that significant improvements in "ability to leave home" ($p = 0.03$), "time spent resting"

($p = 0.03$), and sexually activity ($p = 0.01$) were associated with response to treatment. The researchers also compared results from responders to those of nonresponders. Responders were classified as those who experienced a reduction in 24-hour stool weight of 50% on day 7. From those who completed the questionnaire (74%), the improvement in QOL was statistically significant in favor of the responders ($p = 0.024$) (Koch et al., 1999).

After these studies, a product standardized to contain 250 mg SP-303 per 350-mg tablet (SB-300) was made available in the United States as a dietary supplement known as NSF/Normal Stool Formula™ (Shaman Pharmaceuticals, Inc., South San Francisco, CA) (Carlson et al., 2000).

Viral infections

Herpes simplex. Orozco-Topete et al. (1997) conducted a Phase II clinical study of SP-303 in the treatment of genital herpes simplex virus (HSV) infections in 45 patients with AIDS 20–54 years of age. The purpose of the multicenter, placebo-controlled, double-blind study was to determine efficacy and safety of an ointment (Virend®, Shaman Pharmaceuticals, Inc.) containing 15% SP-303 w/w in the treatment of recurrent anogenital or genital herpes in AIDS patients. Primary endpoints of "complete healing" and "time to healing" of herpes lesions were evaluated in HSV active-phase, culture-positive patients who topically applied an ointment containing SP-303 or a matching placebo (ointment base, twice daily for 21 days). Patients received instructions to cleanse the lesions with mild soap and water and gently blot them dry before covering the lesions with a thin layer of the ointment, once in the morning, afternoon, and evening. At each visit to the clinic, specimens were obtained for cultures of HSV to determine changes in viral positivity. All patients were positive for HSV-2. Blood and serum chemistry were monitored as were vital signs. Lesions were measured and photographed at day 1 when patients were randomized and at each visit. Only one patient was not taking some kind of anti-infective drug therapy during the trial. The agents taken were typical of the population and included antibiotics, antiretrovirals, antituberculosis drugs, sulfonamides, vitamin B complex, and various medicines used to treat nausea, diarrhea, and constipation. Patient withdrawals from the study consisted of 7 of 21 in the placebo group and 5 of 24 in the active treatment group. Reasons for discontinuation were advancing HIV (1 placebo and 2 Virend), burning sensation at the site of application (1 Virend), death (1 placebo and 1 Virend), herpes zoster (1 placebo), concomitant drug therapy (1 placebo), treatment failure (1 placebo), wasting syndrome (1 Virend), esophagitis (1 Virend), patient refusal (2 placebo), and other (1 placebo and 1 Virend) (Orozco-Topete et al., 1997).

Apart from a burning sensation at the site of application (2 Virend and 1 placebo), no other adverse events were thought to be attributable to Virend. In the resolution of lesions at day 21, the results were significant in favor of Virend ($p = 0.053$) only when the two patients lost to follow-up in the Virend group were excluded. On day 21, lesions were completely healed in 41% of the Virend group and only 14% of the placebo group. However, in this small trial, when the two patients lost to follow-up were included in an intent-to-treat analysis, the results failed to reached statistical significance ($p = 0.077$). It is important to note that those who showed complete healing of lesions had both significantly higher levels of $CD4^+$ cells and significantly smaller lesions at the start of the trial (each $p = 0.03$). The number who showed decreases in lesion size ($\geq$25%) was also not significant compared to placebo (25% Virend versus 24% placebo), an outcome the investigators attributed to several Virend patients with large lesions that were only slightly improved. No significant difference was found in lesion pain compared to placebo, although there was a trend towards significance in pain intensity in the Virend group on day 4. As for HSV-2 shedding, 19% of the placebo group became culture-negative during the trial versus 50% of the Virend group ($p = 0.06$). The investigators concluded that the results were not superior to those obtainable using available oral agents (e.g., acyclovir) and planned to conduct further studies using a more easily dissolving formulation of SP-303 (Orozco-Topete et al., 1997).

Inflammatory skin conditions

Insect bites. The potential soothing effect of a 1% *sangre de drago* balm (Zangrado Bug Bite Balm, Rainforest Phytoceuticals, LLC) on itching and pain caused by insect bites was studied in 10 workers from the Terminex Pest Control Company in New Orleans, LA. The balm base served as the placebo and both preparations were coded. Over a period of 3 months, workers applied the preparations at their discretion to various conditions, recording the length of time before they experienced relief, the number of reapplications, if any, and the causes of the skin afflictions. Fire ant bites became the most common affliction and affected all 10 participants. Apart from immediate pain, the bite of fire ants is known to cause an intense itch and the itching can last for weeks. Half of the participants reported pain, 40% discomfort, 60% swelling, 60% redness, and 100% itching. In all instances the number of workers who preferred the active balm over the placebo balm was significant (itching, $p < 0.001$; swelling, $p < 0.01$; and pain, redness, or discomfort, each $p < 0.05$). The average time reported by the workers before symptomatic relief after applying the active balm was less than 2 minutes. These results provided further evidence that *sangre de drago* inhibits sensory nerve afferent activity. Such an inhibitor could potentially relieve any skin condition attended by pain, edema, redness, discomfort, itching, or pain (Miller et al., 2001).

Pharmacokinetics

In eight healthy adult men, "little or no" gastrointestinal absorption and subsequent uptake in the bloodstream was found from oral administration of SP-303 in delayed-release tablets (1250 mg oral single-dose and 500 mg four times per day for 8 days) (Carlson et al., 2000, citing Carlson and Khandwala[§]). Another study in 6 healthy men found that SP-303 was not absorbed into the bloodstream, and in children and in infants 3 months of age or older, SP-303 was also not absorbed into the bloodstream (Carlson and King, 2000, citing Connor et al.,[¶]).

DOSAGE

The traditional internal dosage of the sap in Ecuador and Peru is generally 5–10 drops, once to twice per day for 5 days. Often the treatment is repeated for as long as 3 weeks. The sap is taken in water (cold or warm), milk, or alcohol (Ubillas et al., 1994).

The proprietary product SB-300, NSF/Normal Stool Formula™ is used to promote normal stool formation and for relief from occasional diarrhea. It can be taken for both acute and chronic forms of diarrhea of various origins. Each 350-mg tablet is standardized to contain 250 mg or 67% by weight of the oligomeric procyanidin preparation SP-303. At minimum, 40% of the remaining constituents are composed of unidentified polyphenolic compounds, some of which may also possess antidiarrheal (antisecretory) activity. SB-300 is taken at the suggested daily dosage of 350–700 mg, twice per day to four times per day. However, subjects with irritable bowel syndrome or HIV-associated diarrhea may require long-term use of the formulation (Carlson and King, 2000).

SAFETY PROFILE

Contraindications

Contraindications for SB-300 (NSF/Normal Stool Formula™) are as yet undetermined and none were found in 10 patients diagnosed with diarrhea and HIV (Carlson and King, 2000, citing Koch et al., 2000). In a placebo-controlled trial, absorption of lamivudine, nelfinavir or zidovudine (single doses) was not affected by SP-303 at a dosage of 500 mg four times per day

[§]Carlson T, Khandwala A. Investigator's Brochure for SB-300. South San Francisco, CA: Shaman Pharmaceuticals, May 24, 1999, unpublished.

[¶]Conner JD, Rodriguez W, Englund J. Evaluation of Provir (SP-303) for use in infants and children. South San Francisco, CA: Shaman Pharmaceuticals, 1995, unpublished.

(=2,000 mg/d) (Carlson and King, 2000, citing Porter et al.[‖]).

Drug interactions

No drug interactions from either the latex or SP-303 have been reported. In a placebo-controlled study of SP-303 in HIV-positive patients, the absorption of anti-HIV agents (lamivudine, nelfinavir, or zidovudine) was not affected by the compound when taken at a dosage of 500 mg four times per day for a total daily dose of 2000 mg. SP-303 was well-tolerated (Carlson and King, 2000 citing Porter et al.[‖]).

Pregnancy and lactation

No studies were found on the safety of *sangre de drago* (*C. lechleri* and *C. palanostigma*) or extracts thereof in pregnant or lactating people or animals.

Side-effects

No reports of side effects from internal use of the sap in traditional medicine were found (Ubillas et al., 1994).

Special precautions

Some members of the Euphoriaceae family contain tumor-promoting diterpene (phorbol) esters (Blumberg, 1988); however, these are not found in detectable quantities in the sap of *sangre de drago* (*C. lechleri*, *C. palanostigma* [= *C. draconoides*], and *C. erythrochilus*) (Vlietinck et al., 1995).

Toxicology in vitro

In Chinese hamster V-79 lung fibroblasts, the alkaloid constituent taspine (IC_{50} 0.17 μg/mL) showed potent growth-inhibiting/cytotoxic activity. The sap itself, obtained from Peruvian *C. palanostigma*, also showed activity against the growth of V-79 cells (IC_{50} 3.7 μg/mL). In the KB (human oral epidermoid carcinoma) cell assay, potent cytotoxic activity was also found from taspine (IC_{50} 0.39 μg/mL) (Itokwa et al., 1991). Cytotoxicity against KB cells was absent in tests of crude sap collected from *C. lechleri* growing in Ecuador (IC_{50} 900 μg/mL). The dried sap (IC_{50} 187 μg/mL), various solvent extracts of the sap, and the major constituents of the sap (procyanidins and flavonols) also showed no evidence of cytotoxicity in this assay. This sap contained only traces of taspine (Chen et al., 1994).

Given the cytotoxicity of taspine, the authors of the latter study have recommended that *sangre de drago* saps containing a high content of the alkaloid not be used for wound-healing or for internal use (Chen et al., 1994). Evidence from *in vitro* tests indicates that the precaution is prudent; however, it remains for *in vivo* studies to determine whether the toxicity of taspine is ameliorated by other constituents in the sap and by how much. According to the manufacturing specification for the dietary supplement NSF (Normal Stool Formula™) and NSF-IB (Normal Stool Formula-Ion Balanced), the level of taspine is not to exceed the limit of 5000 ppm) (S.R. King, written communication, November 13, 2001).

Mutagenicity

The raw latex obtained from *C. lechleri* growing in Ecuador showed no mutagenicity in the Ames test, with or without S9 activation (Rossi et al., 2003). SP-303 also showed no mutagenic activity in the Ames test and in Chinese hamster ovary (CHO) cells failed to induce chromosomal aberrations (with or without metabolic activation). Negative results were found in the rat bone marrow micronucleus test (Carlson and King, 2000, citing Carlson and Khandwala[§]).

Toxicity in animal models

In a long-term study (17 months), topical application of the sap derived from *C. lechleri* in the two-stage mouse skin carcinogenesis sys-

[‖]Porter SB, Santos O, Charney MR, Pennington J. A phase I, randomized, double-blind, placebo-controlled interaction study to evaluate the effect of multiple doses of SP-303 or placebo on the pharmacokinetics of the antiviral drugs zidovudine, lamivudine, and nelfinavir in healthy subjects. South San Francisco, CA: Shaman Pharmaceuticals, May 8, 1998, unpublished.

[§]Carlson T, Khandwala A. Investigator's Brochure for SB-300. South San Francisco, CA: Shaman Pharmaceuticals, May 24, 1999, unpublished.

tem resulted in no carcinogenic effects. Taspine was also devoid of carcinogenicity in this test system (Vaisberg et al., 1989).

Toxicity studies of SP-303 in various species of animals found no deaths from single oral doses of up to 300 mg/kg. Neither were there any changes in body weights or food consumption and no clinical indications of toxicity were evident upon examination. The acute oral LD_{50} of SP-303 in the rat was determined to be greater than 300 mg/kg. In repeated-dose toxicity studies of SP-303, the no-observable-effect-level (NOEL) in rats after 30 days of oral administration was greater than 200 mg/kg per day and less than 500 mg/kg per day. In monkeys, the NOEL after 30 days oral administration was greater than 30 mg/kg/day and less than 100 mg/kg per day (Ubillas et al., 1994).

CONCLUSIONS

The results of *in vitro* and *in vivo* studies largely support the majority of ethnomedical uses of the sap. Pending the development of clinically efficacious preparations, *sangre de drago* has the potential of becoming a readily sustainable medicinal resource of financial benefit to the indigenous peoples of northwest Amazonia and therapeutic benefit to the world. Acknowledgement of the diversity in the chemical makeup of the sap from one geographic area to another and the recently proposed alkaloid chemotypes of *C. lechleri* will require that materials being developed for clinical use are consistent after standardization to a chemical profile providing known quantities of one of more active constituents. Phytochemical investigations clearly indicate that the standardization process will involve sourcing and standardized processing of consistent plant material. Recent clinical studies of products prepared from the sap in treatments of diarrhea and symptoms of insect bites have shown positive results that are likely to lead to further research. The pain- and itch-relieving activity of the sap may lead to the development of a substitute for capsaicin, the topical use of which is limited because of its characteristic burning sensation. Clinical research on the topical use of SP-303 against genital and anal herpes lesions in HIV-positive patients yielded results of borderline significance, either because of the small number of patients enrolled, poor solubility of the preparation used, significant differences in the $CD4^+$ cell counts of the subjects, or a combination of factors. The need for less costly treatments of these infections, combined with the unresolved clinical efficacy of SP-303 against herpes, may entice larger, better controlled studies utilizing preparations with greater absorbability. Although the relative toxicity of the content of taspine in *sangre de drago* requires some additional evaluation, the sap has shown low toxicity and preparations used in clinical studies were well-tolerated. Larger trials involving oral and topical preparations of *sangre de drago* are warranted.

The author and many of the researchers whose studies are cited herein are grateful to the peoples of the northwest Amazon basin for sharing their intellectual and medical achievements and the teachings of their healers on *sangre de drago* along with numerous other South American medicinal plants.

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