Sweetgum: An ancient source of beneficial compounds with modern benefits

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ABSTRACT

Sweetgum trees are large, deciduous trees found in Asia and North America. Sweetgum trees are important resources for medicinal and other beneficial compounds. Many of the medicinal properties of sweetgum are derived from the resinous sap that exudes when the outer bark of the tree has been damaged. The sap, known as storax, has been used for centuries to treat common ailments such as skin problems, coughs, and ulcers. More recently, storax has proven to be a strong antimicrobial agent even against multidrug resistant bacteria such as methicillin-resistant *Staphylococcus aureus*. In addition to the sap, the leaves, bark, and seeds of sweetgum also possess beneficial compounds such as shikimic acid, a precursor to the production of oseltamivir phosphate, the active ingredient in Tamiflu®–an antiviral drug effective against several influenza viruses. Other extracts derived from sweetgum trees have shown potential as antioxidants, anti-inflammatory agents, and chemopreventive agents. The compounds found in the extracts derived from sweetgum sap suppress hypertension in mice. Extracts from sweetgum seeds have anticonvulsant effects, which may make them suitable in the treatment of epilepsy. In addition to the potential medicinal uses of sweetgum extracts, the extracts of the sap possess antifungal activity against various phytopathogenic fungi and have been effective treatments for reducing nematodes and the yellow mosquito, *Aedes aegypti*, populations thus highlighting the potential of these extracts as environment-friendly pesticides and antifungal agents. The list of value-added products derived from sweetgum trees can be increased by continued research of this abundantly occurring tree.

Key words: Anti-inflammatory, antimicrobial, antioxidant, *Liquidambar*, storax, sweetgum

INTRODUCTION

Liquidambar is a genus of trees, commonly called sweetgum, which derives its name from the brown sap produced by these trees which, if left to harden, can be made into chewing gum. This deciduous tree is recognized by the quarter sized, spiny, woody seedpods it produces. There are four species of sweetgum including *Liquidambar orientalis* L. (*L. orientalis*), *Liquidambar formosana* Hance (*L. formosana*), *Liquidambar styraciflua* L. (*L. styraciflua*), and *Liquidambar acalycina* (*L. acalycina*) worldwide.

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However, only *L. styraciflua* is native to North America where it has a widespread distribution across the southeastern United States. It grows as far north as Connecticut, ranges south up to Florida, and west as far as Texas. The trees also grow in Central America from Mexico to Panama.[1] Sweetgum trees grow at a moderate rate and can reach heights of 65-70 feet (20 meters), some in excess of 100 feet (30 meters). The trees have straight trunks, and the crown is pyramid-shaped ranging more than 40-60 feet wide (12-18 meters) that becomes more oval as the tree ages. The leaves of the sweetgum tree are palmate with five lobes and are green in the spring and summer but turn an attractive purple, red, or yellow in the fall. Their brilliant fall colors, moderate growth rate, and resistance to most pests make the ornamental sweetgum trees a desirable choice for many landscapes or parks. However, the tree's spiny seedpods can litter lawns in the fall making mowing difficult. The subspecies *L. styraciflua* 'Rotundiloba' is an exception, as it does not produce seedpods. The roots of a sweetgum tree have also been known to lift sidewalks if planted too closely.[2]

Although sweetgum trees have attracted widespread use in landscaping, most sweetgum trees are found growing as a volunteer species among commercial pine forests that are

cultivated for the softwood lumber and pulpwood industries. Sweetgum trees can grow in a wide range of soil types but they grow best in bottomland areas.[3] Sweetgum trees spread quickly and will re-sprout from stump or root after they have been cut.[4] After logging or burning, the sweetgum sprouts can reach a height of nearly 5 feet within a year, whereas sweetgum seedlings take three to five years to reach the same height.[5] Sweetgum trees, along with other hardwoods, such as oaks (*Quercus* ssp.), elms (*Ulnus* spp.), maples (*Acer* spp.), and dogwoods (*Cornus* spp.) are considered to be competitive species to pines, as they grow interspersed with pines, and therefore, present a problem for the softwood lumber and pulpwood industry. Most logging operations require that these competitive species be removed before the pines can be harvested. Nationwide, the cost for removal or elimination of these competitors is approximately 150 million dollars annually, mainly for herbicide treatments.[6] To minimize these costs, a considerable amount of research has shown it is feasible to use these competitive hardwood species for alternative economical uses such as biomass, cellulosic ethanol production, and phytochemical or medicinal co-products. This review focuses on the medicinal properties of sweetgum extracts as well as their beneficial, non-medical applications.

MEDICINAL PROPERTIES OF SWEETGUM

Many of the medicinal properties of sweetgum come from storax as well as essential oils extracted from the leaves. Storax, also referred to as styrax, is produced by damaging the outer bark of sweetgum trees. When the tree is wounded, the inner bark produces a balsam. Boiling the inner bark in water effectively removes the balsam and produces storax. Storax produced from *L. orientalis*, or Turkish sweetgum, is referred to as Asian storax while storax derived from *L. styraciflua* is called American storax. Storax has medicinal uses dating back to the Aztec Empire during the Paleo-Indian Period (ca. 10,000-7000 BC). The ancient Aztecs collected the boiled down, grayish-brown, sticky, opaque liquid and used it as a treatment for skin infections and other ailments.[1] Native Americans also used storax for medicinal purposes, including controlling coughs and dysentery and treating sores and wounds.[7] In addition to storax, the sap of the sweetgum tree was burnt as incense or mixed with tobacco leaves as a sedative[1] as well as used in the making of soaps, cosmetics, fixatives in perfumes, adhesives, and lacquers. Recent references from organic websites have noted that the inner bark of sweetgum, boiled with milk, can relieve diarrhea, and oils from the leaves of sweetgum trees have antimicrobial properties against both bacteria and viruses.[8]

Antibacterial

Asian storax was first reported to contain cinnamic acid, cinnamyl cinnamate, 3-phenlypropyl cinnamate, ethyl cinnamate, benzyl cinnamate, styrene, and vanillin as major constituents while American storax was reported to contain styrene, cinnamic acid, cinnamyl alcohol, 2-phenylpropyl alcohol, 3-phenylpropyl cinnamate, cinnamyl cinnamate and vanillin.[9] Later reports on the constituents may include or omit some compounds but most report the presence of cinnamic derivatives.[10]

Cinnamic acid has known antimicrobial and antioxidant properties.[11] Therefore, it is not surprising that storax has antibacterial activity against several pathogens. Sağdic *et al.,*[12] compared the antimicrobial properties of storax produced from *L. orientalis* at concentrations from 0.1% to 10% against 20 different strains of bacteria using an agar diffusion method. Wells 4 mm in diameter were cut into nutrient agar, which had been inoculated with 1% of a test pathogen. To the wells, 50 μl storax dissolved in ethanol at 0.1%, 0.2%, 0.4%, 1%, or 10% was added. The plates were subsequently incubated at the optimal temperature for each bacterium for 18-24 h, and zones of inhibition were measured. No antibacterial activity was observed at 0.1% concentration. When the storax concentration was increased to 0.2% or 0.4%, the plates containing the Gram-negative bacteria *Proteus vulgaris* (*P. vulgaris*) and *Enterobacter aerogenes* (*E. aerogenes*) showed small zones of inhibition (6-8 mm) indicating that these bacteria were susceptible to storax. At 1%, Gram-positive bacteria *Bacillus cereus* (*B. cereus*) and *Bacillus subtilis* (*B. subtilis*) as well as the Gram-negative bacterium *Pseudomonas fluorescens* (*P. fluorescens*) were also inhibited. Several other bacteria were inhibited by storax when the concentration was raised to 10% including Gram-positive bacteria *Bacillus brevis, Corynebacterium xerosis, Enterococcus faecalis* (*E. faecalis*), *Micrococcus luteus, Myobacterium smegmatis*, and *Staphylococcus aureus* (*S. aureus*), and Gram-negative bacteria *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Among the bacteria that were resistant to storax even at its highest concentration were Gram-positive *Bacillus amyloliquefaciens*, *Bacillus megaterium*, and *Listeria monocytogenes*as well as Gram-negative bacteria *Aeromonas hydrophila, Escherichia coli (E. coli)* including O157:H7, and *Yersinia enterocolitica. B. cereus* showed the greatest susceptibility to storax with a zone of inhibition of 16 mm at 10% concentration. These results clearly demonstrate the antimicrobial properties of storax derived from *L. orientalis* against both Gram-positive and Gram-negative bacteria.

In addition to storax, the leaves of the sweetgum tree are also believed to possess antimicrobial properties. Leaf oil from *L. orientalis* and *L. styraciflua* contained high levels of terpinen-4-ol, α-terpineol, α-pinene, and sabinene as well as other compounds.[13-15] Terpinen-4-ol, is the active ingredient found in the essential oil of the *Melaleuca alternifolia* (Australian tea tree). Tea tree oil has a well-established reputation as an antimicrobial agent[16,17] suggesting that terpinen-4-ol derived from the leaves of *L. styraciflua* and *L. orientalis* may also possess similar antimicrobial properties.

Oskay and Sari[18] studied an ethanol extract from the leaves of *L. orientalis* as well as extracts from 18 other Turkish medicinal plants for their antimicrobial properties *in vitro*. Using an agar diffusion method where 100 μl of extract at a concentration of 4 mg/ml in ethanol was added to the well, it was observed that the leaf extract of *L. orientalis* was an effective antimicrobial against

several strains of pathogenic bacteria with zones of inhibition ranging from 10 mm to 34 mm. The affected bacteria include *P. vulgaris* (zone of inhibition = 34 mm), *P. fluorescens* (22 mm), *Salmonella typhimurium* (22 mm), *S. aureus* (20 mm), as well as multidrug-resistant *S. aureus* (MRSA, 20 mm), *E. aerogenes* (18 mm), *Serratia marcescens* (18 mm), *B. subtilis* (14 mm), *Micrococcus luteus* (12 mm), *E. coli* (12 mm), *Staphyococcus epidermis* (10 mm), *E. faecalis* (10 mm), and *Enterobacter cloaceae* (10 mm). Only *B. cereus* and yeast, *Candida albicans*, were not inhibited by the *L. orientalis* extract. Interestingly, *B. cereus* was the most susceptible bacterium to storax from *L. orientalis*[12] but was unaffected by the leaf oil. However, the chemical composition of leaf oil from *L. styraciflua* differed significantly from the chemical composition of storax produced from the same tree.[14]

In addition, the authors determined that the antibiotic-resistant bacteria MRSA and *E. coli* were more sensitive to the extracts of *L. orientalis* and several other plant species such as *Rosmarinus officinalis* L. (rosemary) than to traditional antibiotics such as penicillin G (10 IU/disk) and vancomycin (30 μg/disk) as determined by a disk diffusion assay, suggesting that the extracts utilize a different mode of action against bacteria than antibiotics. Therefore, the antimicrobial properties of *L. orientalis* and other active plant extracts were further explored by determining the minimum inhibitory concentrations (MICs) and minimum bactericidal concentration (MBC) against MRSA and *E. coli* using a broth macrodiluton method. The *L. orientalis* leaf extract was added to the test broth and serial two-fold dilutions were made. The final volume was 4.5 ml to which 0.5 ml of a bacterial suspension containing 8 $\log_{10}$ colony forming units/ml was added. The tubes were incubated at 37°C for 24 h, after which they were visually inspected for bacterial growth. The lowest concentration of extract that prevented visible growth of the bacterium was considered the MIC. Ten microliters of the remaining negative growth tubes were then subcultured, and the lowest concentration showing no growth or the growth of only one colony was considered the MBC. The lowest concentration at which the *L. orientalis* extract could inhibit MRSA was 8 mg/ml, and at 17.4 mg/ml no viable MRSA was observed in the subcultures. The MIC for *E. coli* was 14.2 mg/ml and the MBC was 22.2 mg/ml. Based on the MBC and MIC data, the leaf extract of *L. orientalis* was one of the most inhibitory extracts of all the plant extracts studied. Only extracts from *Cornus sanguinea* (*C. sanguinea*, common dogwood plant) had similar MIC and MBC values against MRSA (8 mg/ml and 16.6 mg/ml), but *C. sanguinea* was ineffective against *E. coli*. *Eucalyptus camuldulensis* and *Vitis vinifera* (common grape vine) had MIC values of 8.6 mg/ml and MBC values of 14–15 mg/ml against MRSA but were only slightly inhibitory towards *E. coli*. *Ecballium elaterium* (squirting cucumber) was effective against *E. coli* (the MIC of 11.8 mg/ml and MBC of 23.6 mg/ml) but not against MRSA. These results further illustrate the powerful antimicrobial properties of *Liquidambar*.

Antiviral

Between 2009 and 2010, approximately 16,000 people died as a result of contracting the human pathogenic strain of swine influenza termed H1N1.[19] The virus responsible for the H1N1 outbreak and other influenza viruses are susceptible to the antiviral drug Tamiflu®. The key ingredient in Tamiflu® is oseltamivir phosphate, of which shikimic acid is a precursor. Chinese star anise was the initial source of shikimic acid used for the production of this drug; however, much of the shikimic acid now manufactured is generated by a shikimic acid producing *E. coli*, although some still comes from plant sources.[20-22] Sweetgum trees, mainly *L. styraciflua*, were found to contain shikimic acid in their leaves,[23] bark,[24] and young seeds.[25]

Seed pods of *L. styraciflua*, containing both fertile and aborted seeds, were extracted with 65°C water, treated with activated charcoal, filtered, and concentrated. The addition of isopropanol produced a white precipitate. The solution was subsequently boiled, filtered, and concentrated to yield clear yellow syrup, which was chromatographed with 20 ml water and 125 ml 25% acetic acid. The acetic acid fraction was concentrated to produce pure shikimic acid with an average yield of 3.23% w/w from the dry mass of seeds. When the seeds were separated into fertile and infertile seeds and subject to extraction, the fertile seeds returned only 0.14% w/w shikimic acid whereas the yield from infertile seeds was 6.5% w/w.[25] Li *et al.*,[26] also observed that young seeds have a higher concentration of shikimic acid than older seeds, seed hulls, stems, or bark.

Shikimic acid was also extracted from the bark of *L. styraciflua* using methanol (Soxhlet method) or water heated to 65°C or 85°C and compared to the yields of shikimic acid derived from star anise using the same extraction methods. The amount of extracted shikimic acid was quantified by high-pressure liquid chromatography (HPLC). Yields of shikimic acid from star anise were 8.73 ± 2.18 mg/g for the Soxhlet method, 7.27 ± 0.21 mg/g for the 65°C water extraction and 6.37 ± 0.88 mg/g for the 85°C water extraction. The yields from the sweetgum bark were much lower at 1.37 ± 0.21 mg/g, 1.68 ± 0.12 mg/g, and 1.16 ± 0.014 mg/g for the Soxhlet method, 65°C water extraction, and 85°C water extraction, respectively.[24] Although yields of shikimic acid are low compared to those of star anise, the sweetgum trees remain a valuable source of this important medicinal compound.

In addition to possessing valuable shikimic acid, sweetgum extracts have also shown to directly inhibit the H1N1 virus by inhibiting the neuraminidase activity (NA) of the virus. Neuraminidase is involved in virus replication and may facilitate infection;[27] it is also the target for oseltamivir and other current and developing antiviral drugs. The anti-NA of a water extract of the fruits of *L. formosana* and 438 other traditional Chinese medicines was evaluated *in vitro*. The compound 2'-(4-methyl umbelliferyl)-α-D-N-acetylneuraminic acid (MU-NANA) was used as a substrate for the NA enzyme. The enzyme was first incubated with the extracts for 30 min prior to the addition of MU-NANA. An uninhibited enzyme cleaves MU-NANA to produce a quantifiable fluorescent product. Of the 438 extracts tested, only five showed significant inhibitory activity against

NA derived from the H1N1 virus including the extract from *L. formosana* (IC_{50} of 102.2 μg/ml). Against NA derived from *Clostridium perfringens*, extracts had an IC_{50} value of 1.0 μg/ml. In comparison, the control quercetin had IC_{50} values of 5.4 and 0.6 μg/ml for NA derived from the H1N1 virus and *C. perfringens*, respectively.

The same five extracts which suppressed NA *in vitro* were evaluated for their ability to inhibit the influenza A H1N1 virus in a cell culture model. The extracts were incubated with the virus for 2 h, serially diluted, then added to Madin-Darby canine kidney (MDCK) cells followed by another 2-h incubation. Concentrations of the extracts ranged from 0 to 250 μg/ml. The cells were then washed and cultured an additional three days and examined for damage to the host cell due to virus invasion (cytopathic effect, CPE) using an assay to measure cell viability. Virus yield was determined by measuring NA in cell supernatants. All five extracts could reduce the CPE of the virus and NA in a dose-dependent manner. When the virus alone was added to the cells, the CPE reduced cell viability to 25%; addition of 200 μg/ml of the extract of *L. formosana* increased cell viability to 50%. At this same concentration of extract, the relative activity of the virus was reduced to 25%.[28] Thus, sweetgum trees are valuable resources for antiviral medications in that they contain shikimic acid and extracts that effectively inhibit the H1N1 virus.

Antioxidant

Many chronic diseases such as Alzheimer's disease and atherosclerosis as well as cancer have been linked to oxidative damage caused by the generation of reactive oxygen species (ROS). Antioxidants are reducing agents that remove ROS and other free radicals by donating an electron or hydrogen atom to make the free radical more stable and thus inhibiting further oxidation reactions. Antioxidants synthesized within the body help to curb the damage done to cells by ROS; however, the synthesized amounts are often insufficient and exogenous sources of antioxidants are required. Three species of *Liquidambar, L. formosana*, *L. styraciflua*, and *L. orientalis* have been shown to possess antioxidant potential.

Phenolic and flavonoid compounds were extracted from the leaves of *L. formosana* using ethanol, water, or acetone and tested for their antioxidant activity by measuring their free radical-scavenging ability as well as their reducing power (ability to donate electrons). A phosphomolybdenum assay was also used to measure antioxidant activity. The stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS; converted to its radical cation by the addition of potassium persulfate) were used to measure the free radical-scavenging ability. The free radical DPPH is purple and turns clear or pale yellow upon neutralization, while ABTS radical cation is blue and becomes colorless upon neutralization. The free radical-scavenging ability of *L. formosana* leaf extracts was performed by adding varying concentrations of the leaf extract with DPPH or ABTS and monitoring absorbance at 515 nm for DPPH and 734 nm for ABTS.

The reducing power of the extracts was measured by their ability to reduce ferricyanide (Fe^{+3}) to ferrocyanide (Fe^{+2}). Ferrocyanide is subsequently detected by the addition of ferric chloride to form a brilliant blue ferrous complex. Varying concentrations of the extracts were added to a 1% ferricyanide solution and incubated at 50°C for 20 min, upon which 10% trichloroacetic acid was added, the mixture was centrifuged, and ferric chloride was added to the supernatant. The absorbance was monitored at 700 nm, at which the increase in absorbance correlated to an increase in reducing power.

The phosphomolybdenum assay measures the reduction of Mo (VI), in the form of ammonium molybdate, to produce a phosphate/Mo (V) complex at acidic pH. The extracts were combined with a reagent solution consisting of ammonium molybdate, sodium phosphate, and sulfuric acid, incubated at 95°C for 150 min, and allowed to cool to room temperature. The formation of the green phosphate/Mo (V) complex was detected by measuring absorbance at 695 nm.

Results of the radical-scavenging ability and reducing power of *L. formosana* extracts were reported as EC_{50} values (half-maximal effective concentration, EC_{50}), in which the EC_{50} value correlates to the concentration at which 50% of the maximal effect was observed. In general, the radical-scavenging ability and reducing power increased as the concentration of the extracts was increased. Ethanol extracts were better at reducing DPPH and ABTS radicals than either the water extract or acetone extract. The DPPH EC_{50} values (the concentration where DPPH radicals were scavenged by 50%) were 6.89 μg/ml, 8.86 μg/ml, and 12 μg/ml for ethanol, water, and acetone extracts, respectively. All extracts were significantly better at scavenging DPHH compared to the widely used synthetic antioxidant butylated hydroxytoluene (BHT). BHT is a powerful antioxidant that is often used as a food additive to preserve meats and other products containing fats or oils. The EC_{50} value for BHT scavenging of DPPH radicals was 14.5 μg/ml, nearly twice as high as the ethanol extract of *L. formosana* leaves. Ethanol extracts were also better than water extract, acetone extract, and BHT at scavenging ABTS radicals with EC_{50} values of 3.86 μg/ml, 4.2 μg/ml, 4.5 μg/m, and 8.15 μg/ml, respectively. The reducing power as measured by the ferricyanide assay produced EC_{50} values of 0.632 mg/ml for the ethanol extract, 0.685 mg/ml for the water extract, and 0.891 mg/ml for the acetone extract, which were more effective than synthetic standard BHT that exhibited an EC_{50} value of 0.983 mg/ml. The antioxidant activity of the ethanol extract and water extract were similar based on results of the phosphomolybdenum assay, with a maximum absorbance of 0.6-0.65 after 150 min. The acetone extract was weaker, (maximum absorbance of approximately 0.3) but not as weak as BHT (maximum absorbance of approximately 0.2). All extracts of *L. formosana* possess excellent antioxidant activities, superior to the synthetic antioxidant BHT. The greater antioxidant activity of

the ethanol extracts is attributed to their higher concentration of phenolic and flavonoid compounds, 76.40 mg/g and 18.76 mg/g, respectively, compared to 71.76 mg/g and 19.97 mg/g for water extracts and 63.97 mg/g and 17.44 mg/g for acetone extracts.[29]

The leaf and stem oils isolated from the species *L. styraciflua* also possess antioxidant properties. Leaf and stem oils were isolated by hydrodistillation, and the antioxidant properties were measured using the DPPH radical-scavenging assay, an assay which measures superoxide radical (O_2^-)-scavenging activity, and a deoxyribose degradation assay. Superoxide anion radicals are generated by oxidation of nicotinamide adenine dinucleotide (NADH) by phenazine methosulfate (PMS). The superoxide anion radicals in turn reduce nitro blue tetrazolium (NBT) to a purple-blue formazan compound, which can be detected by measuring absorbance at 560 nm. A decrease in absorbance correlates with increased superoxide-scavenging abilities. Different concentrations of essential oils were added to a solution containing NADH and NBT. The reaction was initiated by the addition of PMS, and absorbance was measured after 5 min.

The deoxyribose degradation assay is a measure of the hydroxyl radical-scavenging ability. Hydroxyl radicals are generated by the reaction of an iron-EDTA complex with hydrogen peroxide in the presence of ascorbic acid. The generated hydroxyl radicals in turn react with 2-deoxyribose (2-DR), which, when heated in the presence of thiobarbituric acid (TBA) at low pH produces a thiobarbituric acid-malondialdehyde complex. Essential oils were incubated with EDTA, $FeCl_3$, H_2O_2, and ascorbic acid for 1 h at 37°C, after which TBA and trichloroacetic acid were added followed by incubation at 100°C for 20 min. Absorbance was measured at 532 nm, and quercetin was used as a positive control.

Results were reported as IC_{50} values (half maximal inhibitory concentration, IC_{50}) or the concentration of a drug in which 50% of the reactive substance is inhibited *in vitro*. The DPPH radical-scavenging ability of the stem oil was slightly better than that of the leaf oil (IC_{50} of 2.19 mg/ml and 3.17 mg/ml, respectively) but was considerably low compared to the natural antioxidant, vitamin C (3.28 μg/ml). The stem oil was also better at scavenging superoxide radicals with an IC_{50} value of 37.34 μg/ml compared to 78.38 μg/ml for the leaf oil but not as effective as vitamin C (IC_{50}, 29.46 μg/ml). IC_{50} values reported for the 2-DR assay were 17.55 μg/ml for the leaf oil and 14.29 μg/ml for the stem oil compared to 4.71 μg/ml for the control antioxidant quercetin. The higher activity of the stem oil over the leaf oil is attributed the presence of more oxygenated metabolites in the stem oil; however, both oils were low in phenolic compounds, which explains their low activity in the DPPH assay compared to the control.[30]

Antioxidant properties have also been found in the bark of *L. styraciflua*. Two methods were used to extract the phytochemicals from the bark including a high temperature (85°C) water extraction method and a sublimation reaction. Products obtained from the sublimation reaction were purified by centrifugal partition chromatography (CPC) and analyzed by HPLC. The antioxidant activity was measured using a thiobarbituric acid reactive substances (TBARS) assay. A TBARS assay measures the amount of lipid peroxidation as the result of oxidative stress. The water extract displayed antioxidant activity at a concentration of 12.5 mg bark/ml water, in that little to no lipids was oxidized after 24 h. HPLC analysis showed that that the crude extract contained gallic acid, which was shown to be a strong antioxidant. Shikimic acid was also present in the crude extract; however, it did not show any antioxidant activity even at concentrations as high as 200 μM. The extract obtained by sublimation reaction showed extremely potent antioxidant activity starting at 0.78% in DMSO. The compounds responsible for the high antioxidant activity in the sublimation extract have not been identified.[31]

A third member of the *Liquidambar* species has also been tested for its antioxidant properties. The resin from *L. orientalis* and seven other Turkish plants underwent steam distillation (SD) or supercritical carbon dioxide ($SCCO_2$) extraction and the resultant products were analyzed by GC-MS and tested for their radical-scavenging activity using the DPPH assay. Both $SCCO_2$ and SD extracts of *L. orientalis* demonstrated high antioxidant properties in that they were able to reduce DPPH free radicals by 89% and 91%, respectively, compared to 91% for the BHT positive control. Only $SCCO_2$ extracts of *Rosmarinus officinalis* L. (rosemary) and *Cuminum cynimum* L. (cumin) extracts exhibited higher radical-scavenging abilities (approximately 92% of DPPH). The main components of both SD and $SCCO_2$ extracts were octyl alcohol acetate (20.12% and 12.42%), *N*-nordextromethorphan (14.38% and 7.19%), 6α, 17β-dihydroxy-5β-androstan-3one (12.87% and 22.92%), and menthol (23.14% and 31.59%). In addition, the $SCCO_2$ extract contained high levels of rimuen (5.82%) while the SD extract had high levels of neocembrene (4.66%), phenanthrene (8.75%). The high antioxidant capacity of *L. orientalis* was attributed to a high concentration of oxygenated monoterpenes and monoterpene ketone groups.[32] Interestingly, neither extract contained high levels of cinnamic derivatives as otherwise reported;[33,34] this may be due to the different methods of extraction, since extractions with $SCCO_2$ contained free fatty acids while extractions with SD did not.[32] With many consumers wanting to avoid synthetic chemicals in their food, a natural plant extract may be promising as an all-natural antioxidant.

Anti-inflammatory

Oxidative stress and ROS has been discovered to trigger pro-inflammatory cytokine production, which eventually leads to inflammation. Additionally, inflammatory reactions are known to produce ROS (reviewed in Mittal *et al.,*[35]) Several diseases are associated with chronic inflammation including atherosclerosis, Crohn's disease, and cancer. Oils from the leaves and stems of *L. styraciflua* have been examined for their anti-inflammatory activity by measuring 5-lipoxygenase (5-LOX) inhibition. Lipoxygenases catalyze the oxygenation of fatty acids, such as linolenic and arachidonic acids. Oxidation of

arachidonic acid is the first step in the synthesis of leukotrienes, a group of lipid pro-inflammatory mediators; therefore, reducing 5-LOX activity may lead to a reduced inflammatory response. Soybean lipoxygenase was combined with the essential oils in phosphate buffer and incubated for 10 min at room temperature. The substrate, sodium linoleate was added and the reaction kinetics were measured at 234 nm every 10 s. Leaf and stem oils were equally effective at 5-LOX inhibition with an IC_{50} value of approximately 24 μg/ml. The control nordihydroguaiaretic acid, a potent LOX inhibitor, produced an IC_{50} of 0.24 μg/ml. Anti-inflammatory properties of *L. styraciflua*. were also measured by a prostaglandin E2 (PGE2) assay in which an enzyme-linked immuonosorbent assay (ELISA) is used to measure the production of the inflammatory mediator, PGE2 in lipopolysaccharide (LPS)-stimulated hepatic cancer cells (HepG2 cells). The addition of stem or leaf oils to LPS stimulated HepG2 cells reduced PGE-2 levels by 39.66% and 26.46%, respectively, compared to cells stimulated with LPS without the addition of oils. Leaf and stem oils were analyzed by GC-MS, and small amounts of β-caryophyllene oxide (0.1% to 0.5%) were found and thought to be responsible for 5-LOX inhibition. In addition, β-caryophyllene and α-humulene, also known as cyclooxygenase-2 (an enzyme involved in inflammation) inhibitors, were also found in leaf and stem oils, which may further increase the anti-inflammatory properties of *L. styraciflua*.[30]

Antihypertensive

Hypertension, commonly known as high blood pressure, is a serious condition that if left untreated may increase the risk of heart disease. Angiotensin II (Ang II) when associated with its Ang II type 1 receptor (AT1) leads to vasoconstriction which in turn leads to increased blood pressure. The development of antagonist receptor blockers to selectively block the action between Ang II and AT1 has become effective treatment for hypertension. Ohno *et al.,*[30] screened 435 plant extracts for antagonism toward Ang II receptors by utilizing a human cell line that stably expressed the AT1 receptor that was pretreated with fura 2-AM, an intracellular fluorescent indicator of Ca^{2+}. When Ang II interacted with AT1, cellular uptake of Ca^{2+} occurred, which could be measured spectrophotometrically. The fura 2-AM pretreated cells were treated with 1 μM Ang II for 200 s in the presence and absence of plant extracts and the Ca^{2+} uptake was monitored. Preliminary studies showed that an ethanol extract from the resin of *L. styraciflua* was able to suppress the Ca^{2+} uptake. Therefore, it was separated into two active compounds, benzyl cinnamate and benzyl benzoate, and these compounds were evaluated for their ability to reduce hypertension. Both compounds were found to not be cytotoxic to cells at 100 μg/ml after 24 h and were found to repress Ang II in a dose-dependent manner with IC_{50} values of 47.2 μg/ml and 107.2 μg/ml, respectively. Losartan, the first Ang II agonist to be marketed (under the trade name Cozaar), was used as a positive control and produced an IC_{50} value of 2.4 μg/ml. Benzyl benzoate was further examined for its *in vivo* activity. Mice were pretreated with 2 mg/kg or 10 mg/kg of benzyl benzoate and injected with 100 μg/kg of Ang II after 30 min to increase blood pressure. Systolic arterial pressure was measured prior to injection and 4 min after Ang II injection. Mice pretreated with benzyl benzoate showed a significant reduction in blood pressure elevation compared to non-treated controls. Blood pressure raised approximately 20–40 mmHg with benzyl benzoate pretreatment compared to a 100 mmHg increase without the pretreatment. The authors also prepared derivatives of benzyl benzoate of which 3-methylbenzyl benzoate and 3-methylbenzyl 2'-nitrobenzoate showed more than 10-fold greater activity on Ang II-stimulated Ca^2 + uptake. Thus, components including benzyl cinnamate and benzyl benzoate found in the resin of *L. styraciflua* may be useful in the treatment of hypertension.[36]

Antihepatotoxic

The liver plays many important roles in the human body including detoxification of chemicals and drug metabolism. Unfortunately, these same chemicals and drugs may cause liver damage, and finding drugs that provide protection to the liver is vitally important. The crude drug "rorotsū" derived from the fruits of *L. formosana* has long be utilized in Taiwan as an antihepatotoxic agent.[37] Therefore, methanol extracts of spiny fruits of *L. formosana* were separated into active constituents and tested for their antihepatotoxic activity *in vitro*. The main components of the extract were bertulonic acid, 28-noroleanonic acid, caryophyllene oxide, (-)-bornyl cinnamate, styracin, and glycyrrhizin. Primary-cultured rat hepatocytes were pre-incubated with selected *L. formosana*-derived compounds at 0.01, 0.1, or 1.0 mg/ml then subjected to carbon tetrachloride (CCL_4) or galactosamine (GalN) to induce cytotoxicity. Cells were then monitored for glutamic-pyruvic transaminase (GPT) activity. GPT belongs to a group of alanine transaminases, which are elevated during liver damage. Only bertulonic acid at 1.0 mg/ml concentration could significantly reduce GPT levels in CCl_4- or GalN-induced cells. Bertulonic acid reduced GPT levels by 68% and 47% in CCl4-damaged and GalN-induced cells, respectively. The antihepatotoxic effect of crude drugs derived from the fruits of *L. formosana* may be explained by the presence of bertulonic acid[37] and may warrant further investigation.

Anticonvulsant

Anticonvulsants are used in the treatment of epilepsy and in recent years have been used in the treatment of bipolar disorder as they have the ability to stabilize patients' moods. Guo *et al.,*[38] evaluated the anticonvulsant and sedative effects of storax from *L. orientalis in vivo*. Mice were treated orally or intranasally with various concentrations (50, 100, or 200 mg/kg orally or 12.5, 25, or 50 mg/kg intranasally) of storax 5, 15, or 30 min prior to an intraperitoneal injection of 90 mg/kg of pentylenetetrazole (PTZ) to induce convulsions. Mice, 10 per group, were monitored for 30 min after PTZ injection for delay of colonic seizure onset as well as the number and duration of the episode and effects on mortality. Intranasal administration of storax offered significant protection from PTZ-induced seizures in a dose-dependent manner. At its highest concentration of 50 mg/kg, the latency time was delayed by approximately 10 min compared to the

non-treated control mice. The mortality rate was also reduced to 10% compared to 60% in the control. Lower doses of 12.5 and 25 mg/kg had little effect on the latency time but they lowered the mortality rate to 40% and 30%, respectively. Orally administered storax at 100–200 mg/kg was effective at reducing the mortality rate (20%) only when administered 30 min prior to PTZ. No effects were observed when storax was administered 5 or 15 min prior to PTZ injection nor were significant delays in colonic seizure onset observed. The effects of nasally administered storax on PTZ-induced convulsions were observed when storax was administered as little as 5 min prior to PTZ; however, no anticonvulsant activity was observed when PTZ was injected 30 min after storax administration suggesting that storax has fast onset of action but a short lasting time. No dose of storax administered orally or nasally had a significant effect on the total incidence of colonic convulsions. Diazepam, marketed as valium, when administered 30 min prior to PTZ exhibited no colonic convulsions, a mortality rate of 0%, and a convulsion rate of 0% in the 30 min of observation post PTZ injection.

To determine the sedative effect of storax, storax was administered intranasally or orally (50, 100, or 200 mg/kg for orally or 12.5, 25, or 50 mg/kg intranasally) followed by monitoring the number of spontaneous locomotor movements in a locomotor-monitoring apparatus. Mice were placed in the box and allowed to habituate for 5 min, after which they were monitored for spontaneous locomotor activity for 1 h. Only storax at 100 mg/kg administered orally had sedative effects as observed by reduced spontaneous locomotor movements, approximately 600 versus 1000 for non-treated control group. These data suggest that intranasal administration of storax may be effective at reducing seizures and aiding in resuscitation, and oral administration of storax may be effective for the treatment of anxiety disorders.[38]

Wound healing

Ocsel *et al.*,[39] studied the effects of storax on wound healing. Wounds were excised on the backs of young Yorkshire pigs and treated with storax obtained from L. *orientalis*, hydrocolloid, or silver sulfadiazine. The wounds were biopsied on days 4, 8, 14, and 21 and examined histologically. After 21 days, storax-treated wounds showed faster re-epithelialization, as evidenced by a thicker epithelial layer compared to the non-treated control group and hydrocolloid group. Epithelial thickness was similar to wounds treated with silver sulfadiazine, which is extensively used to treat burns. The enhanced epithelialization of oriental storax is thought to be due to the presence of cinnamic acid and its esters cinnamyl cinnamate and storesinol (cinnamic acid ester of an alcohol). Granulation tissue was also thicker in storax-treated wounds compared to that in the control-, hydrocolloid-, or silver sulfadiazine-treated groups suggesting a more vigorous proliferation phase in these wounds treated with storax.[39]

Anti-ulcerogenic

Just as storax is able to treat wounds and skin ulcers, it is also effective in treating gastric ulcers. Gurbuz *et al.*,[40] studied the ulcer preventing activity of storax *in vivo*. Storax in 0.5% Tween 80 and distilled water was orally administered to Sprague-Dawley rats at a dose of 150 mg/kg and 300 mg/kg of body weight, respectively. Thirty minutes after administration of the storax, 1 ml of absolute ethanol was applied orally to induce gastric ulcers. After 1 h, the animals were sacrificed and the stomach removed and examined for blood clots to assess the number of ulcers. Storax administered at 150 mg/kg was more effective at reducing gastric ulcer formation with 83% inhibition compared to the non-treated control. At a dose of 300 mg/kg, storax had an ulcer inhibition rate of only 66.7%. Moreover, the lower dose completely prevented ulcers in four out of six stomachs whereas at the higher dose, only one stomach out of five was completely free of ethanol-induced ulcers. Misoprostol, prescribed to treat stomach ulcers, was used as a positive control at 0.4 mg/kg and showed 100% inhibition of ethanol-induced ulcers. To further investigate the anti-ulcerogenic potential of storax, it was extracted with chloroform and *n*-butanol and water. The chloroform extract exerted significant protection against ethanol-induced ulcers (61.5% ulcer inhibition rate) but prevented ulcers only in one out of six mice, whereas the *n*-butanol and water extract only had an inhibition rate of 10.3% and did not prevent ulcer formation. Volatile oils of storax and its extracts were obtained by microdistillation and analyzed by GC and GC-MS. Thirty-one essential oils of storax were identified and the main components were styrene (81.9%), cinnamyl alcohol (6.9%), α-pinene (3.5%), and benzene propanol (3.4%). The chloroform extract contained 27 volatile compounds of which styrene was present in the highest amount (75.5%) Other compounds included cinnamyl alcohol (7.3%), benzene propanol (3.8%), β-pinene (2.4%), and α-pinene (1.8%). The *n*-butanol and water extract contained only 17 identifiable compounds including styrene (72.8%), (*E*)-methyl cinnamate (5.9%), (*E*)-cinnamaldehyde (3.2%), cinnamyl alcohol (3.0%), and benzene propanol (2.0%). Although styrene was present in all samples, it may not be the only compound responsible for the anti-ulcerogenic results since the *n*-butanol and water extract contained 72.8% styrene but showed an inhibition rate of only 10%. Nonetheless, storax as a whole is effective in the prevention of ulcers.

Cancer chemopreventive

Phytochemicals for the prevention and treatment of cancer have been used throughout history, and a number of common plant and vegetables have reported chemopreventive properties.[41] Fukuda *et al.*,[42] isolated 11 compounds from the seedpods of *L. styraciflua* and tested them for their anticancer activity using an Epstein–Barr early antigen (EBV-EA) *in vitro* assay. The Epstein–Barr virus is a known oncogenic virus and is activated by tumor promoters such as12-*O*-tetradecanoylphorbol-13-acetate (TPA) to produce early antigens (EA). The EBV-EA–expressing cells are detected from serum obtained from nasopharyngeal carcinoma (NPC) patients by staining so that the serum contains the antibodies to EBV-EA, which are detected by indirect immunofluorescence. Studies were performed in Raji cells, which are lymphoblastoids carrying the EBV genome. Cells were incubated with butanoic acid (a co-inducer) and TPA (32 pmol in DMSO) along with

test compounds at concentrations of 1000, 500, 100, and 10 mol ratio/TPA in DMSO. Smears were made from cell suspension, and activated cells were detected by staining by EVB-EA-containing positive serum from NPC patients and detected by indirect immunofluorescence. Control cells that were not treated with any test compounds were assumed to have 100% activation of EBV-EA. All compounds exhibited dose-dependent inhibitory activities but compounds 2 (3β,25-expoxy-3α-hydroxylup-20 (29)-en-28-oic acid) and 3 (3β, 25-epoxy-3α-hydroxyolean-12-en-28-oic acid) exhibited inhibitory activities higher than those of the positive control, curcumin. Compound 2 was able to inhibit TPA induced EBV-EA activation by 100%, 81.3%, 31.7%, and 8% at concentrations of 1000, 500, 100, and 10 mol ratio/TPA, respectively, while compound 3 showed inhibition rates of 100%, 80.5%, 32.1%, and 8% for the same respective concentrations. The known anti-tumor promoting agent, curcumin inactivated TPA induced EBV-EA TPA activation by 100%, 77.2%, 18.3%, and 0% at 1000, 500, 100, and 10 mol ratio/TPA, respectively. Compound 1 (25-acetoxy-3α-hydroxyolean-12-en-28-oic acid) showed inhibitory activity, (inactivation rates of 93%, 74.7%, 27.6%, and 2.7%) although not as strong as compound 2 or 3. However, previous reports indicated that compound 1 was cytotoxic to 39 different human cancer cell lines.[43] Therefore, further studies were pursued with compounds 1 and 2, which examined the *in vivo* effects of the compounds in reducing the incidence and onset of papillomas induced on the backs of mice.

Mice were treated topically with the tumor initiator 7,12-dimethylbenz[*a*] anthracene (DMBA; 390 nmol) to initiate mutagenesis. One week following DMBA application, mice were treated twice weekly with TPA (1.7 nmol) to promote tumor formation. Test compounds (85 nmol) were applied 1 h prior to TPA application, and the incidence of papilloma formation was monitored for 20 weeks. Compound 1 delayed the onset of papillomas for 8 weeks with an average number of 4.1 papillomas per mouse at week 20 compared to the control group which saw papillomas as early as week 6 and averaged 9.3 papillomas per mouse at week 20. Compound 2 delayed the onset of papilloma formation for 8 weeks with the average number of tumors being 4.2 at week 20. Similar results were observed with the positive control, curcumin. Compounds 1 and 2, in addition to having similar *in vivo* chemopreventive activities as curcumin, are similar in structure to oleanolic acid and betulinic acid both of which have demonstrated *in vitro* anticancer activities[44,45] suggesting that these compounds may be valuable agents for cancer prevention.

Compounds extracted from the resin of *L. formosana* also show promise as anticancer agents based on cytotoxicity studies. Yang *et al*.,[46] used petroleum ether followed by separation by repeated silica gel chromatography to extract 10 pentacyclic triterpenes, two of which were previously unknown, from the resin of *L. formosana*. These 10 triterpenes were evaluated for cytotoxicity against a human breast carcinoma cell line using a thiazolyl blue tetrazolium bromide (MTT) assay. Living cells reduce the yellow MTT in their mitochondria to form purple formazan crystals which, when solubilized in DMSO, can be measured spectrophotometrically. Cells were seeded in 96-well plates, and after 24 h the test compounds were added at varying concentrations. Adriamycin, a drug used in cancer chemotherapy, was used as a positive control. Cells were cultivated for an additional 72 h followed by the addition of 5 mg/ml MTT per well. After 4 h, the media was removed and DMSO was added to the wells to dissolve the formazan crystals; the absorbance of the resulting solution was measured at 490 nm. Seven of the 10 compounds exhibited strong cytotoxicity towards the tumor cells (IC_{50} values ranging from 3.06 μg/ml to 13.82 μg/ml), although none were as effective as Adriamycin (IC_{50}, 0.33 μg/ml), a drug commonly used in chemotherapy treatments. Comparison of the 10 compounds showed that compounds containing a 3-keto group (3-keto oleane triterpenes) were important antitumor agents.

Anticoagulant

The 10 pentacyclic triterpenes evaluated for cytotoxicity against human breast cancer cells were also evaluated for their anticoagulative properties. Blood was collected from New Zealand white rabbits, mixed with anticoagulant, and separated into platelet-rich and platelet-poor plasma by centrifugation. Test compounds were added to the platelet-rich plasma which had been adjusted to 3×10^5 with platelet-poor plasma. The samples were incubated at 37°C for 1 min, and 10 μM of adenosine diphosphate was added to stimulate platelet aggregation. The turbidity of the solution as a result of clotting was measured using an aggregometer. Aspirin was used as a positive control. Only four compounds showed significant reduction in platelet aggregation with IC_{50} values ranging from 8.07 μg/ml to 13.05 μg/ml; these values were similar to that of the positive control aspirin which had an IC_{50} value of 10.59 μg/ml. Compounds that contained a 28-carboxyl group (28-carboxyl oleane triterpenes) were effective inhibitors of platelet aggregation and may be effective in preventing heart attacks and strokes.[46]

Immunosuppression

The immune system exists to protect the body from infection. However, conditions such as rheumatoid arthritis, inflammatory bowel disease, and other autoimmune diseases are characterized by hyperactivity of the immune system wherein the body's defense system attacks its own tissues. In these cases, it is necessary to suppress the immune system to alleviate symptoms; as in the case of organ transplant, suppression of the immune system will reduce the chances that the organ will be rejected. The nuclear factor of activated T-cells (NFAT) is a transcription factor important for immune response, and modulation of NFAT is thought to be a treatment for autoimmune diseases. Cyclosporine A (CsA) is a current immunosuppressant used for transplant recipients but often has adverse side effects including being carcinogenic; therefore, safer alternatives are being sought including those from plant sources such as the fruits of *L. formosana*. Fruits were dried, ground, extracted with methanol, and concentrated after which they were suspended in water and partitioned with *n*-hexane, ethyl

acetate, and butanol and chromatographed to isolate specific compounds. A modified secreted alkaline phosphatase (SEAP) assay was used to measure their inhibitory activity against NFAT. An oligonucleotide containing the NFAT binding site was subcloned into a SEAP vector, which was subsequently transfected into Jurkat cells. Jurkat cells are T-cells that express NFAT. Once the vector is established in the cells, the expressed NFAT will bind to the NFAT-binding site on the vector allowing for expression of SEAP. A substrate for SEAP is then added to the cell supernatant, which converts it into a substrate that can be detected by chemiluminescence or fluorescence once acted upon by SEAP. The ethyl acetate extract along with four compounds isolated from the extract were added to cells containing the NFAT/SEAP vector. CsA was used as a positive control for NFAT suppression. After incubation for 18 h at 37°C, the supernatant was removed from the cells and the SEAP substrate was added. The SEAP substrate and cell supernatant containing SEAP were incubated at 37°C for 4 h, and the optical density was measured at 405 nm. The ethyl acetate extract demonstrated strong NFAT suppression with an IC_{50} value of 4.31 μg/ml. Two of four isolated oleanane triterpenoids, lantanolic acid (IC_{50} of 12.62 μM), and 3a-acetoxy-25-hydroxy-olean-12-en-28-oic acid (IC_{50} of 4.63 μM) also showed strong inhibitory activity. None were as effective as CsA (IC_{50} of 0.29 μM). Although these compounds show immunosuppressive activity, more research on their safety is necessary before they can be considered for use in humans.[47]

OTHER USES OF SWEETGUM

Antifungal

In addition to its antimicrobial properties, extracts derived from the sweetgum trees also have the ability to reduce fungal growth. Fumigant antifungal activity of an essential oil obtained from the resin of *L. orientalis* against common phytopathogenic fungi including *Phytophthora cactorum*, *Cryphonectria parasitica*, and *Fusarium circinatum* were evaluated by Lee *et al.,*[34] Agar plates were inoculated in the center with selected fungi by insertion of a mycelial agar plug. Essential oils were added to a paper disk which was placed on the lid of the plate. The plates were incubated upside down in the dark for 6-8 days depending on the type of fungus. The final concentrations of *L. orientalis* essential oil were 0.028, 0.017, 0.007, and 0.0035 mg/ml air. At its highest concentration (0.028 mg/ml air), *L. orientalis* essential oil was highly effective at inhibiting *P. cactorum*, (100% inhibition) compared to essential oils from other plants which showed 60% inhibition or lower. At the same concentration, *L. orientalis* essential oils inhibited growth of *C. parasitica* by approximately 40% and *F. circinatum* by 45%. Lowering the concentration to 0.017 mg/ml air lowered inhibition rates to 74% and 38% for *P. cactorum* and F. *circinatum*, respectively; *C. parasitica* was not inhibited at this concentration. At concentrations of 0.007 and 0.0035 mg/ml air, *L. orientalis* essential oil was only inhibitory towards *P. cactorum* at 60% and 41%, respectively. Gas chromatography analysis of the *L. orientalis* essential oil revealed the major components to be hydrocinnamyl alcohol and *trans*-cinnamyl alcohol. These as well as cinnamyl aldehyde, acetopheneone, and benzaldehyde, compounds also found in sweetgum essential oils, were further tested for their antifungal activity. Only cinnamyl aldehyde at the highest concentration exhibited inhibitory activity to *F. circinatum* (28% inhibition at 0.028 mg/ml air) and was highly inhibitory towards *P. cactorum* (100%) even at low concentrations (0.86×10^{-3} mg/ml air). Acetophenone was the only compound to inhibit *C. parasitica* (35% inhibition at 0.028 mg/ml air) but was ineffective against other fungi. Benzaldehyde also showed 100% inhibition toward *P. cactorum* at 0.028 mg/ml air while hydrocinnamyl alcohol and cinnamyl alcohol showed moderate activity (50% and 47%, respectively) at the same concentration. Scanning electron microscopy of *P. cactorum*, treated with sweetgum essential oil or its components cinnamyl aldehyde or benzaldehyde, showed significant morphological changes and alteration of hyphae suggesting that the essential oils interfere with wall synthesis. In all, essential oils from the oriental sweetgum may be effective treatments for phytopathogenic fungi especially *P. cactorum*, which may eventually replace synthetic fungicides that are often toxic to the environment and humans.[34]

Compounds from the balsam of *L. formosana* were found to be inhibitory to two wood decay fungi, *Lenzites betulina (L. betulina)*, a type of white rot fungi, and *Laetiporus sulphureus (L. sulphureus)*, a brown rot fungus. The balsam was first fractionated using *n*-hexane and ethyl acetate and separated by HPLC. A total of 26 compounds were isolated and nine of the compounds in highest abundance were tested against *L. betulina* and *L. sulphureus* on potato dextrose agar plates. The compounds at 30 μg/ml or the unfractionated balsam at 50, 100, or 200 μg/ml were added to agar and inoculated with select fungi. The plates were then incubated at 27°C until the mycelium of the control plate had reached the edge of the dish. The diameter of the growth zone was measured for test substances and compared to the growth zone of the control dish to calculate the antifungal index (AI). Two compounds showed strong antifungal activity. Bornyl cinnamate was able to suppress the growth of *L. betulina* and *L. sulphureus* with AI of 70% and 46%, respectively, whereas AI for 3α,25-dihydroxyolean-12-en-28-oic acid was 62% and 63%, respectively. The IC_{50} for the unfractionated balsam was 159.7 μg/ml and 181.1 μg/ml for *L. betulina* and *L. sulphureus*. These data further emphasize the antifungal properties of sweetgum resin and its extracts.[48]

Pesticide

Essential oil obtained from the resin of *L. orientalis* has shown nematicidal activity against a common pine wood nematode, *Bursaphelenchus xylophilus*. Nematodes were treated with the essential oil in various concentrations in a 96-well plate for 24 h, after which the nematodes was monitored microscopically and defined as dead if they were not moving and had straight bodies. At 2 mg/ml of *L. orientalis* essential oil the mortality of the nematodes was 100%. Reducing the concentration to 0.8 mg/ml or 0.6 mg/ml led to reduced mortality rates of 98% and 86%, respectively. Analysis of the essential oil by GC or GC-MS showed

that its major compounds were hydrocinnamyl alcohol (41%) and *trans*-cinnamyl alcohol (45%). These individual compounds were also tested for their anti-nematidical activity. It was discovered that trans-cinnamyl alcohol effectively killed 100% of the nematodes at a concentration of 2 mg/ml. At this same concentration, hydrocinnamyl alcohol produced a 17% mortality rate. Lower concentration of *trans*-cinnamyl alcohol were studied, and it was found that at concentrations of 1.0, 0.8, and 0.6 mg/ml the mortality of the nematodes was still essentially 100% however, further reduction to 0.4 mg/ml or 0.2 mg/ml decreased mortality to 57% and 11%. At concentrations of 0.6 mg/ml or higher, the essential oil of *L. orientalis* may be effective as a treatment for pine wilt disease caused by the pine wood nematode.[49]

Aedes aegypti (A. aegypti) mosquitoes are known to transmit dengue fever and dengue hemorrhagic fever. No vaccines against this disease are available; therefore, prevention relies on control of *A. aegypti* and protection from its bites. Storax from *L. orientalis* was evaluated as an insecticide to target *A. aegypti* larvae. Healthy larvae were placed in a sterilized plastic bowl without food and with a range of concentrations of storax. After 24 h, the dead and moribund larvae were enumerated. Mortality occurred in a dose-dependent manner with 100, 200, 300, 400 and 500 ppm storax produced 16%, 44%, 75%, 91%, and 99% mortality rates. The LC_{50} value was calculated to be 194.93 ppm. These results suggest that storax from *L. orientalis* may be used to control *A. aegypti* and replace the use of environmentally harmful synthetic chemicals.[50]

CONCLUSIONS

Sweetgum trees, with their straight trunks, pyramid-shaped crowns, and brilliant fall foliage, are desired as an ornamental tree in southeastern United States. However, this prolific species commonly invades managed pine forests and competes with pines for resources. Additionally, sweetgum trees add additional burden to loggers as they have to be cut and hauled away separately during harvest. Research has shown that economically feasible alternatives exist for sweetgum trees including use as biomass, for cellulosic ethanol production, or for extraction of phytochemical co-products for medicinal uses.

Historically, the sap from the sweetgum bark has been utilized for medicinal purposes dating back more than 10,000 years. The sap was also used by Native Americans for treating wounds, coughs, and stomach problems. Ingredients from the sap were also used for cosmetic purposes. Recent reports on the medicinal benefits of sweetgum resin include its effectiveness as an antimicrobial agent, an antioxidant, an anticoagulant, and a cancer chemopreventive agent. It has been shown to expedite wound healing and reduce hypertension in mice. In addition, it was shown to prevent ulcers in rats as well as reduce the number of PTZ-induced convulsions suggesting that it may be beneficial for treating epilepsy. Aside from its medicinal uses, storax also has potential to be used as an environmentally friendly pesticide as well as an antifungal agent against plant pathogenic fungi.

Beneficial compounds have also been discovered in the leaf, bark, and fruit extracts of sweetgum including shikimic acid, which is a precursor to oseltamivir phosphate-a potent antiviral agent. Leaf extracts are reported to have antioxidant properties *in vitro* as well as anti-inflammatory activity with low cytotoxicity. Extracts from the fruits are potential cancer chemopreventive agents and may help reduce liver toxicity. Additionally, they may act an immunosuppressant agent for patients with autoimmune diseases and prevent organ rejection for transplant patients without adverse side effects. The list of value-added products derived from sweetgum trees can be increased by continued research of this abundantly occurring tree, which may prove to be a valuable resource in southern forests.

REFERENCES

1. Virginia Tech Non-timber Forest Products Fact Sheet no. 19. 2001. Available from: http://www.ntfpinfo.us/docs/other/VirginiaTech2001-SweetgumFactsheet.pdf [Last cited on 2008 Nov 10].
2. Gilman EF, Watson DG. *Liquidambar styraciflua* Sweetgum. US Forest Service Fact Sheet ST-358. Available from: http://hort.ufl.edu/database/documents/pdf/tree_fact_sheets/liqstya.pdf [Last accessed on 2014 Feb 11].
3. Kormanik PP. *Liquidambar styraciflua* L. sweetgum. In: Burns RM, Honkala BH, editors. Technical Coordinators. Silvics of North America. Hardwoods; Agriculture Handbook 654. Vol. 2. Washington DC: US Department of Agriculture, Forest Service; 1990. p. 400-5. 877.
4. Wenger KF. The sprouting of sweetgum in relation to season of cutting and carbohydrate content. Plant Physiol 1953;28:35-49.
5. Mercker D. Short Rotation Woody Crops for Biofuels, University of Tennessee Biofuels Initiative, University of Tennessee Extension Publication (SP702-C). Available from: http://trace.tennessee.edu/cgi/viewcontent.cgi?article=1010 and context=utk_agexbiof [Last accessed on 2014 Mar 4].
6. Siry JP. Intensive timber management practices. In: Wear DN, Greis JG, editors. Southern forest resource assessment. Southern Research Station, Technical Report GTR SRS-53, Ashville, North Carolina; 2002. p. 327-40.
7. Moerman DE. Native American Ethnobotany. Portland, Oregon: Timber Press; 1998. p. 927.
8. Lost Creek Witch.com Sweetgum. 2008-2009. Available from: http://www.lostcreekwitch.com/98/sweetgum/ [Last cited on 2014 Feb 22].
9. Guenther E. Oil of Styrax. The Essential Oils. Vol. 17. New York: D. Van Nostrand Company Inc.; 1952. p. 507.
10. Lawrence BM. Progress in essential oils. Perfum Flav 2007;32:44-6.
11. Sova M. Antioxidant and antimicrobial activities of cinnamic acid and derivatives. Mini Rev Med Chem 2012;12:749-67.
12. Sağdic O, Ozkan G, Ozcan M, Ozcelik S. A study on inhibitory effects of Sigla Tree (*Liquidambar orientalis* Mill. var. *orientalis*) storax against several bacteria. Phytother Res 2005;19:549-51.
13. Tattje DH, Bos R, Bruins AP. Constituents of essential oil from leaves of *Liquidambar styraciflua* L. Planta Med 1980;38:79-85.
14. Wyllie SG, Brophy JJ. The leaf oil of *Liquidambar styraciflua*. Planta Med 1989;55:316-7.
15. Duru ME, Cakir A, Harmandar M. Composition of the volatile oils isolated from the leaves of *Liquidambar orientalis* Mill. Var. *orientalis* and *L.orientalis* var. *integriloba* from Turkey. Flavour

Frag J 2002;17:95-8.

16. Cox SD, Mann CM, Markham JL, Gustafson JE, Warmington JR, Wyllie SG. Molecules 2001;6:87-91.
17. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) Oil: A review of antimicrobial and other medicinal properties. Clin Microbiol Rev 2006;19:50-62.
18. Oskay M, Sari D. Antimicrobial screening of some Turkish medicinal plants. Pharm Biol 2007;45:176-81.
19. World Health Organization. Evolution of a pandemic A (H1N1) 2009. In: Rashford A, editor. 2nd ed. Available from: http://apps.who.int/iris/bitstream/10665/78414/1/9789241503051_eng.pdf [Last accessed on 2014 Jan 6].
20. Bradley D. Star role for bacteria in controlling flu pandemic? Nat Rev Drug Discov 2005;4:945-6.
21. Krämer M, Bongaerts J, Bovenberg R, Kremer S, Müller U, Orf S, *et al*. Metabolic engineering for microbial production of shikimic acid. Metab Eng 2003;5:277-83.
22. Johansson L, Lindskog A, Silfversparre G, Cimander C, Nielsen KF, Lidén G. Shikimic acid production by a modified strain of *E. Coli* (W3110.shik1) under phosphate-limited and carbon-limited conditions. Biotechnol Bioeng 2005;92:541-52.
23. Plouvier V. On the study of quinic and shikimic acids in certain Anacardiaceae. C R Hebd Seances Acad Sci 1960;250:1721-3.
24. Martin E, Duke J, Pelkki M, Clausen EC, Carrier DJ. Sweetgum (*Liquidambar styraciflua* L.): Extraction of shikimic acid coupled to dilute acid pretreatment. Appl Biochem Biotechnol 2010;162:1660-8.
25. Enrich LB, Scheuermann ML, Mohadjer A, Matthias KR, Eller CF, Newman MS, *et al*. *Liquidambar styraciflua*: A renewable source of shikimic acid. Tetrahedron Lett 2008;49:2503-5.
26. Li S, Yuan W, Wang P, Zhang Z, Zhang W, Ownby S. United States Patent Application Publication. Pub. No. US 2007/0161818 Al. Pub. Date: 2007 Jul. 12.
27. An J, Lee DC, Law AH, Yang CL, Poon LL, Lau AS, *et al*. A novel small-molecule inhibitor of the avian influenza H5N1 virus determined through computational screening against the neuraminidase. J Med Chem 2009;52:2667-72.
28. Tian L, Wang Z, Wu H, Wang S, Wang Y, Wang Y, *et al*. Evaluation of the anti-neuraminidase activity of traditional Chinese medicines and determination of the anti-influenza A virus effects of the neuraminidase inhibitory TCMs *in vitro* and *in vivo*. J Ethnopharmacol 2011;137:534-42.
29. Wang K, Pan Y, Wang H, Zhang Y, Lei Q, Zhu Z, *et al*. Antioxidant activities of *Liquidambar formosana* Hance leaf extracts. Med Chem Res 2010;19:166-76.
30. El-Readi MZ, Eid HH, Ashour ML, Eid SY, Labib RM, Sporer F, *et al*. Variations of the chemical composition and bioactivity of essential oils from leaves and stems of *Liquidambar styraciflua* (Altingiaceae). J Pharm Pharmacol 2013;65:1653-63.
31. Hurd S. Sweetgum bark: Extraction, purification, and determination of antioxidant activity. Masters Thesis, University of Arkansas, Department of Biological and Agricultural Engineering, Arkansas, USA; 2012.
32. Topal U, Sasaki M, Goto M, Otles S. Chemical compositions and antioxidant properties of essential oils from nine species of Turkish plants obtained by supercritical carbon dioxide extraction and steam distillation. Int J Food Sci Nutr 2008;59:619-34.
33. Hafizoglu H. Analytical studies on the balsam of *Liquidambar orientalis* Mill. by gas chromatography and mass spectrometry. Holzforschung 1982;36:311-3.
34. Lee YS, Kim J, Lee SG, Oh E, Shin SC, Park IK. Effects of plant essential oils and components from Oriental sweetgum (*Liquidambar orientalis*) on growth and morphogenesis of three phytopathogenic fungi. Pestic Biochem Phys 2009;93:138-43.
35. Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. Antioxid Redox Signal 2014;20:1126-67.
36. Ohno O, Ye M, Koyama T, Yazawa K, Mura E, Matsumoto H, *et al*. Inhibitory effects of benzyl benzoate and its derivatives on angiotensin II-induced hypertension. Bioorg Med Chem 2008;16:7843-52.
37. Konno C, Oshima Y, Hikino H, Yang LL, Yen KY. Antihepatotoxic principles of *Liquidambar formosana* fruits. Planta Med 1988;54:417-9.
38. Guo J, Duan JA, Tang Y, Li Y. Sedative and anticonvulsant activities of styrax after oral and intranasal administration in mice. Pharm Biol 2011;49:1034-8.
39. Ocsel H, Teke Z, Sacar M, Kabay B, Duzcan SE, Kara IG. Effects of oriental sweet gum storax on porcine would healing. J Invest Surg 2012;25:262-70.
40. Gurbuz I, Yesilada E, Demirci B, Sezik E, Demirci F, Baser KH. Characterization of volatiles and anti-ulcerogenic effect of Turkish sweetgum balsam (Styrax liquidus). J Ethnopharmacol 2013;148:332-6.
41. Gullett NP, Ruhul Amin AR, Bayraktar S, Pezzuto JM, Shin DM, Khuri FR, *et al*. Cancer prevention with natural compounds. Semin Oncol 2010;37:258-81.
42. Fukuda Y, Sakai K, Matsunaga S, Tokuda H, Tanaka R. Cancer chemopreventive activity of lupane- and oleanane-type triterpenoids from the cones of *Liquidamber styracifl*ua. Chem Biodivers 2005;2:421-8.
43. Sakai K, Fukuda Y, Matsunaga S, Tanaka R, Yamori T. New cytotoxic oleanane-type triterpenoids form the cones of *Liquidamber styraciflua*. J Nat Prod 2004;67:1088-93.
44. Jeong HG. Inhibition of cytochrome P450 2E1 expression by oleanolic acid: Hepatoprotective effects against carbon tetrachloride-induced hepatic injury. Toxicol Lett 1999;105:215-22.
45. Tan Y, Yu R, Pezzuto JM. Betulinic acid-induced programmed cell death in human melanoma cells involves mitogen-activated protein kinase activation. Clin Cancer Res 2003;9:2866-75.
46. Yang NY, Chen JH, Zhou GS, Tang YP, Duan JA, Tain LJ, *et al*. Pentacyclic triterpenes from the resin of *Liquidambar formosana*. Fitoterapia 2011;82:927-31.
47. Dat NT, Lee IS, Cai XF, Shen G, Kim YH. Oleanane triterpenoids with inhibitory activity against NFAT transcription factor from *Liquidambar formosana*. Biol Pharm Bull 2004;27:426-8.
48. Chien SC, Xiao JH, Tseng YH, Kuo YH, Wang SY. Composition and antifungal activity of balsam from *Liquidambar formosana* Hance. Holzforschung 2013;67:345-51.
49. Kim J, Seo SM, Lee SG, Shin SC, Park IK. Nematicidal activity of plant essential oils and components from coriander (*Coriandrum sativum*), oriental sweetgum (*Liquidambar orientalis*), and valerian (*Valeriana wallichii*) essential oils against pine wood nematode (*Bursaphelenchus xylophilus*). J Agric Food Chem 2008;56:7316-20.
50. Imam H, Riaz Z, Sofi G. Mosquito larvicidal efficacy of storax (*Liquidambar orientalis*) against *Aedes aegypti* L. larvae. J Nat Remedies 2013;13:104-8.

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