REFERENCES

PAPER MULBERRY
Broussonetia papyrifera

1. **Paper Mulberry and its preparations as Tyrosinase Inhibitors and Skin Lightening Agents**

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Abstract

Paper Mulberry (*Broussonetia kazinoki* Siebiold or *B. papyrifera* Vent. Tabl. Veget. or hybrids of both) root was extracted via different methods to achieve the optimum extraction for tyrosinase activity, inhibition. The optimum extract is compared to other inhibitors to determine the inhibitory concentration required to cause 50% reduction in tyrosinase activity (IC_{50}).

A proprietary blend (Albacan) war prepared containing, in addition Bearberry leaf dry extract and a debridement enzrme together with well-selected cofactors, activators and stabilizers, in order to achieve maximal activity and eliminate side effects. The testings, carried out by a reputed independent lab, are quite promising.

Introduction

A great number of skin lightening products have been developed recently and are becoming increasingly popular in Fast-Asian countries, as well as in Africa and South America. North America and Europe have also shown increased interests in skin lightening agents. Asian and dark-skinned people we skin lightening produce to whiten, even-tone or brighten their skin: while for white-skinned population the purpose is even-toning and brightening. Skin lightening products can also be used to treat pigmentation disorders such at age spots, freckles and pregnancy masks. The most popular skin lightening agents in common use are: Kojic acid, Kojic dipalmitate, arbutin, hydroquinone and magnesium ascorbyl phosphate. Our goal is to develop a skin lightening product using a plant extract, which would have the same or even better effect than the mentioned chemical agents. Plant products would be natural, safer and less irritant. Extraction or Paper Mulberry root was selected and compared to Uva-ursi leaf extract and proprietary blends with co-factors, activators and stabilisers, in order to supply the consumer with the safest, effective product.

Results and Discussion

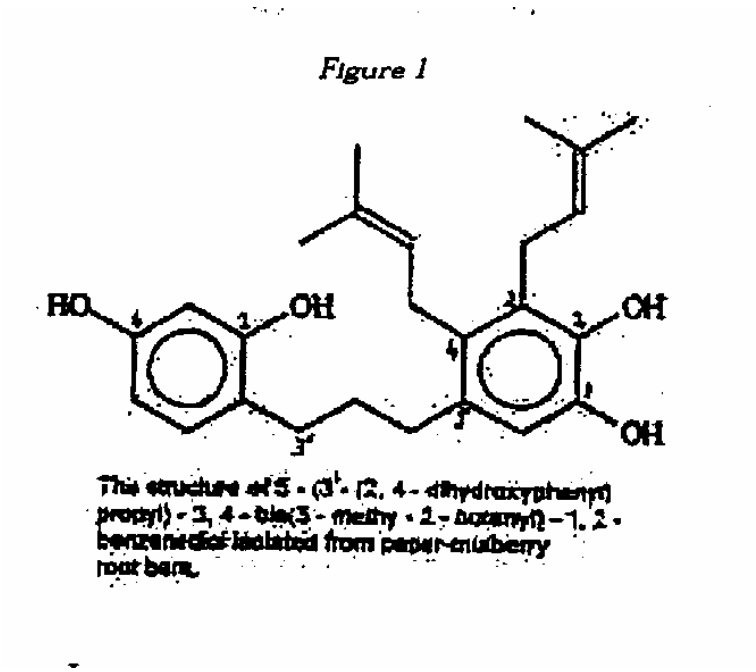
Paper Mulberry is obtained from *Broussonetia kazinoki* Siebold *B. papyrifera* Vent. Tabl. Regn. Veget. or hybrids of both, family Moraceae, which is commonly found in many parts of the world. Extracts of the root bark of the plant are potent inhibitors of tyrosinase enzyme (1). The plant itself is a highly branching tree with a longitudinally wrinkled stem showing transverse lenticels. Leaves are ovate to ovate-lanceolate, margin dentate to crenate, upper surface dark green and rough due to abundant unicellular prickly hairs carried on small multicellular dome-shaped protuberances, lower surface is wooly due to uniseriate multicellular (2 to 3-celled) covering trichomes abundant over the veins. Young apical leaves

are 2 to 5-palmately lobed. The roots are brown longitudinally striated. Odour faint; taste woody and characteristic (1).

For extraction, the whole coarsely powdered root together with the bark was used. Different extracting menstruums were tested, monitored by HPLC analysis and the optimum menstruum was determined.

The major active constituent of Paper Mulberry root bark has been successfully isolated and its structure was determined by UV, IR, H-NMR, C-NMR and Mass spectrometries (2). The chemical structure is shown in Fig 1.

Figure I



It is of utmost importance to properly identify every root lot and compare it to the Voucher sample kept at Bio-Botanica. Inc. Herbarium. Figure 2 shows the HPLC chromatogram of an authentic roof extract, as compared to an allied root of commerce.

The authentic root extract shows the main compound at 9,400 min., while the allied root extract shows high concentrations of different metabolites end only a small concentration of the required compound.

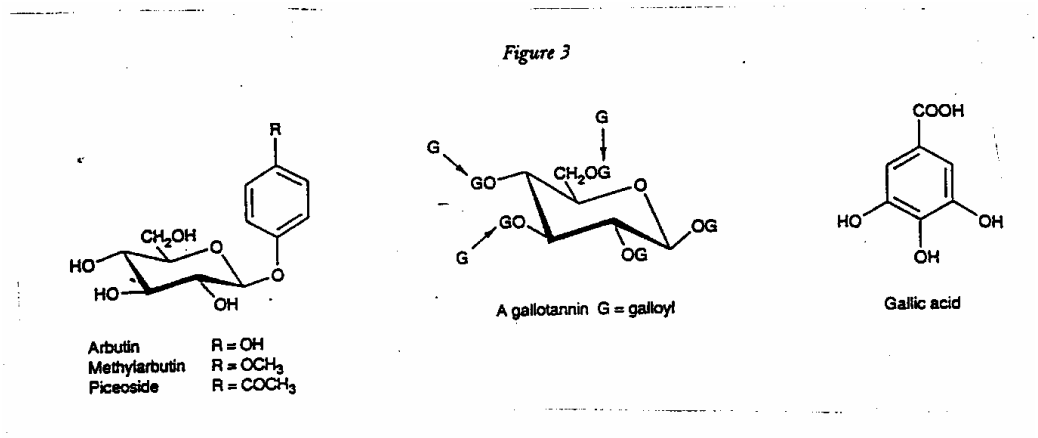
The crucial and first step in melanin formation is the oxidation of the amino acid tyrosine to dopaquinone catalyzed by copper-containing tyrosinase. The rest of the reaction sequence proceeds spontaneously at physiological pH. A compound which inhibits tyrosinase activity would hinder melanin formation. Tyrosinase activity is usually analyzed by spectrophotometry using the method described by Vanni et al (3). In this test the concentration of Paper Mulberry compound causing 50% inhibition of tyrosinase activity (IC_{50}) was much lower than that of kojic acid, ascorbic acid or Hydroquinone.

According to Jang, et al (2). Paper Mulberry Compound was effective in *in vivo* guinea pig depigmentation test and showed no primary irritation and sensitization potential on human skin and in rabbit eye - irritation tests. (Please note: Paper Mulberry and other products prepared at Bio-Botanica. Inc. are not subjected to any animal testing).

According to the previous results, an extract of Paper Mulberry containing 5.0% of the active compound would show a quire potent lightening effect when used as low as 0·1-1% concentration in the final product.

Although the use of Paper Mulberry extract alone is quite effective and without any noticeable side effects, we advise to use a special proprietary blend using even smaller amounts of each ingredient to achieve the desirable effect in a smoother, more tolerable way devoid of any irritation or sensitization.

The suggested blend would contain in addition Bearberry Leaf (*Arctostaphylos uva-ursi*) Spreng.. Ericaceae) extract, which contains natural glycosides of hydroquinone, mainly arbutin and methyl arbutin together with small amounts or piceoside, a gallotannln and gallic acid. (Figure 3, page 34).



A third ingredient, which is present in the suggested blend, is a minimal effective concentration of a debridement enzyme together with the necessary co-factors, activators and stabilizers, which might also have a potentiating effect on the main lightening ingredients.

Table 1

Concentration causing 50% inhibition of mushroom tyrosinase of common inhibitors as compared to Paper Mulberry compound (2)

Material	**IC_{50} (μg/ml)**
Paper Mulberry Compound	0.306
Hydroquinone	5.500
Kojic acid	10.00
Ascorbic acid	70.00

The suggested proprietary blend has a trade name **Albacan**, which has been registered by Bio-Botanica. Inc. - It contains:

· Dried standardised extract of Paper Mulberry Root
· Dried standardised extract of Bearberry leaf
· Debridement enzyme
· Co-factors, Activators. Stabilizers

This blend is parent pending, waiting for the final results of reputed independent lab testings. A very reliable testing protocol using the *in vitro* Melanoderm (Mat-Tek) model of human epidermis based on the work of Majmudar et al (4) is applied.

At the time of writing this report the final results were still unavailable, however, a quick but promising preliminary statement was obtained, spelling exactly as follows:

“The skin whitening test is complete. The visuals look very good with your product comparing well to the 1% Kojic acid. The negative controls ‘darkened up’ considerably. I have sent the tissues out for histophotography as well as a melanin assay. I’ll advise you when the reports are back.”

As soon as the final results are obtained they will be conveyed to the magazine for publication in the next issue. as an addendum.

Literature cited

1. F.S. D'Amello. Sr. in 'Botanicals a Phytocosmetic Desk Reference' p 167. CRC Press. Boca Raton - London - New York, Washington. D.C. (1999).
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4. G. Majmudar, G. Jacob, Y. Laboy and L. Fisher. J. Cosmet. Sci. 49, 361 (1998).

About the Authors

Frank S. D'Amelio. Sr. has over 31 years experience in the botanical industry He is the founder and CEO of Bio-Botanica and is an associate referee on botanical drugs for the Association of Analytical Chemists. He is the author of a number of published articles and most recently a book. 'Botanicals: A Phytocosmetic Desk Reference'.

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2. From the Internet. Kapa re-emerges as Hawaiian art form, potential industry in Ka Wai Ola OHA newsletter.

Imagine the days when all of the cloth that was worn as garments, slept under as bedding, used for ceremonial occasions, wrapped around the bones of the ancestors, every imaginable use we now have for loomed fabric...all of this cloth came from the inner bark of trees, the most cultivated and useful of which is wauke, the paper mulberry tree. Wauke is also known as **Broussonetia** papyrifera, and is a member of the fig (Moraceae) family. It is the principle plant used in the making of kapa, tapa cloth. Kapa means the beaten thing. One of the principle plants introduced by early voyaging Polynesians who settled here in Hawai`i, wauke is thought to have been carried in the canoes as root shoots, but can also be grown from cuttings and occasionally by seed.

The ancient practice of making bark cloth and bark paper likely began in Asia, where other species of **Broussonetia** are known, yet in Polynesia, **Broussonetia** papyrifera reigns supreme. The quality of its cloth is prized and valuable, warm, water resistant, long lasting, washable, soft, mothproof, flexible and white. In Polynesia, the loom and weaving of fibers

did not occur, possibly because there were no mammals of size to provide hair; and cotton, flax, hemp and silk were not produced.
Wauke grows best in moist areas, along streams, in forests, wherever soil is rich, and where there is protection from the wind. Usually a small tree or shrub, wauke can grow as tall as 50 feet, but in cultivation, the plants were kept small. Nurtured and carefully tended by both men and women, wauke was grown around the lo`i, taro pond fields, and near home sites in thick stands of 8-9 foot tall trees. Keiki grow from roots of established plants, so the wauke grove extends outwards naturally.

As the wauke tree grew, planters cut off the side branches, so a straight trunk stalk without branch holes could later be stripped. In 6-10 months the trunk shoots were cut down and the roots and tops removed. The trunks were stripped of bark, as thick as a finger and about 4 feet long. The outer bark was slit and peeled off. The inner bark fibers, called bast, were then soaked in running water, such as a high tide pool, with stones placed on top of the fiber pile. This part of the process breaks down the woody fibers and washes away the starch. A complicated process of soakings and fermentation followed, leaving the fine fibers of the moist inner bark still tough and resilient when finally removed from the waters. At this time in the process, the women of Hawai`i would often twist cordage out of the fibers, for use as fish nets, upena, and as carrying nets, koko, from which to hang calabashes of wood and gourds.

The women then spread the strips of inner bark to be used for cloth into several layers, all of the same thickness. After the water was drained away, the fibers began to stick together and the entire bundle could be lifted as one mass. This was called mo`omo`o. It was beaten on smooth rocks and sun dried. Mo`omo`o was then often stored until enough material had accumulated to make a large piece of kapa cloth. When enough mo`omo`o bundles were accumulated, these were soaked for half a day, then pounded gently to loosen the fibers. They were then laid between layers of mai`a, banana leaves, for a week to mature and ferment, making the fibers softer, almost like the leavening process in bread making. These cakes of wauke were then kneaded until elastic. When ready, the women laid the bundle on a long piece of wood with a flat even surface, an anvil called kua kuku. Then the women beat the pulp with a simple heavy mallet, a wooden beater called hohoa, until a solid strip formed. The beating established a rhythm with resonating sounds. After this, the strips were soaked again. The cloth expanded greatly with each beating. It was often doubled over and beaten again.
Most wooden beaters, i`e kuku, used in the final stage, were four-sided, carved and incised with stamps in intricate geometric designs and patterns. The kapa maker left her "watermark" imprinted on the kapa she made. These can be seen by holding the kapa up to the light.
At this point in the process, the cloth was hung in the sun to dry and to bleach whiter. The quality of the kapa varied according to the bark quality and the skills of the maker. Most i`e kuku were made from kolea wood.

After the kapa was completed, intricate designs were added to some of the cloth with dyes and a high quality of colored stamped patterns. These were unique to Hawai`i and are considered a lost art form, although in the last 15 years a few people have resumed the making and stamping of kapa in the old way, renewing this lost art.

The beaters, anvils and stamps were made by the men of the village. The dyes came from varied natural plant sources. Pigmentation comes in many colors, predominantly black, from

burying the cloth in the mud of a kalo lo`i, brown from such as kukui root bark and red from its bark, yellow and orange from the tuber of `olena, red from noni's bark and yellow from its root. Geometric designs with inherent symbolism, such as lines, triangles, circles and chevrons were imprinted using stencils and stamps usually carved in `ohe, bamboo. `Ohe was also used for brushes, as were dried hala drupes (fruit). Sticky adhesives were used to waterproof kihei, capes. Some kapa was scented, usually that which was to be stored. Aromatic oils, seeds and flowers of such as maile, mokihana, `awahuhi, `iliahi/sandalwood, hinano and kamani flowers were applied.

The clothing made was a knee length wrap-around skirt called pa`u (pareau, sarong, lava lava) for women; loin cloth, malo, for men; sandals, hula costumes and the kihei, cape. Bed clothes were kapa moe, single sheets of kapa, placed together for warmth, a modern double-bed size. Also, kapa ku`ina, several layers stitched along one side with wauke cordage, was used in colder climates. The top layer was called kilohana, usually decorated and/or dyed.

There are two existing forms of paper mulberry. Wauke, of superior quality, and po`a`aha, which produces very thin sheets of kapa. The plants are similar, except for the leaves. Those of wauke are thick and rough on top, woolly underneath and are lobed. The leaves of po`a`aha are velvety soft and rounded. The leaves of both plants are heart shaped with serrated edges, and are 4-6 inches long by 3-5 inches wide. The flowers are rare and sparse, fuzzy, one inch round with hairy bracts and long stigmas. Fruit are rare, and are orange and one inch round.
Other sources for kapa fibers are mamake, a species of pipturus, which produces a dark fiber. Ma`aloa also produces brown fibers. Other occasional sources are po`ulu and mamane.

Medicinally, the slimy sap of wauke is a mild laxative. `Ea, thrush, a mouth disease, is said to be improved when the ash from burned kapa made from wauke is applied to the mouth. Kapa figured in several Hawai`i legends, one of which is about the god Maui and his mother, the goddess Hina, and how he lassoed the sun from Haleakala to slow down the day, so it would be warm long enough to dry his mother's kapa cloth.

There were a great abundance of wauke plants grown in Hawai`i prior to the introduction of foreign fabrics, but now this once cherished plant is rarely to be found.

3. **Grieve**, Maud.: A Modern Herbal, 1984 Savvas Publishing. ISBN unknown.

Grieve, Maud: A Modern Herbal – the medicinal, culinary, cosmetic and economic properties, cultivation and folklore of herbs, grasses, fungi, shrubs and trees with all their modern scientific uses. 1998 Tiger Books International, London. ISBN No.1-85501-249-9.

The bast-fibres of many *Moraceae* are tough and are used in the manufacture of cordage and paper. The Paper Mulberry (*Broussonetia papyrifera*, Vint.) is cultivated extensively in Japan. It is a native of China, introduced into Great Britain early in the eighteenth century and is a coarse-growing, vigorous shrub, or a tree up to 30 feet, forming a roundish, spreading head of branches. The young wood is thickly downy, soft and pithy, the leaves very variable in size and form, often shaped like fig-leaves, the upper surface dull, green and rough, the lower surface densely woolly. It is a dioecious plant, the male flowers in cylindrical, often curly, woolly catkins, the female flowers in ball-like heads, producing

round fruits congregated of small, red, pulpy seeds. In Japan, the stems are cut down every winter, so that the shrub only attains a height of 6 or 7 feet, and the barks are stripped off as an important material for paper. *B. Kajinoki* (Sieb.) is a deciduous tree, wild in Japan, growing 29 to 30 feet high, similar to the Paper Mulberry and made use of in like manner, though inferior. The ripe fruits are beautifully red and sweet. Paper is also manufactured in Japan with the fibre of the bark of *B. kaempferi* (Sieb.), a deciduous climber. A good paper may be manufactured from the bast of the *Morus alba*, var. *stylosa* (Bur.), Jap. 'Kuwa,' but as this plant is used especially for feeding silkworms, the paper made from the branches after the leaves are taken off for silkworms is of a very inferior quality.