Tara Gum

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Abstract *— Caesalpinia spinosa* (Molina) Kuntze, commonly known as *tara* (Quechua), is a small leguminous tree or thorny shrub native to Peru. *C. spinosa* is cultivated as a source of tannins based on a galloylated quinic acid structure. This chemical structure has been confirmed also by LC-MS. It is also grown as an ornamental plant because of its large colorful flowers and pods. *C. spinosa* is native to Peru and can be found growing throughout northern, western and southern South America, from Venezuela to Argentina. It has been introduced in drier parts of Asia, the Middle East and Africa and has become naturalized in California. Generally resistant to most pathogens and pests, it will grow between 0 and 3,000 meters above sea level, tolerates dry climates and poor soils including those high in sand and rocks. To propagate, seeds must be scarified (pre-treated to break physical dormancy), and young plants should be transplanted to the field at 40 cm in height; trees begin to produce after 4–5 years. Mature pods are usually harvested by hand and typically sun dried before processing. If well irrigated, trees can continue to produce for another 80 years, though their highest production is between 15 and 65 years of age. Enhancement of tumor cell sensitivity may help facilitate a reduction in drug dosage using conventional chemotherapies. Consequently, it is worthwhile to search for adjuvants with the potential of increasing chemotherapeutic drug effectiveness and improving patient quality of life. Natural products are a very good source of such adjuvants. The biological activity of a fraction enriched in hydrolysable polyphenols (P2Et) obtained from Caesalpinia spinosa was evaluated using the hematopoietic cell line K562. This fraction was tested alone or in combination with the conventional chemotherapeutic drugs doxorubicin, vincristine, etoposide, camptothecin and taxol. The parameters evaluated were mitochondrial depolarization, caspase 3 activation, chromatin condensation and clonogenic activity.

Keywords *- Poinciana spinosa* MOL., *Caesalpinia pectinata* CAV., *C. tara*, *C. tinctoria* HBK, *Coulteria tinctoria* HBK, *Tara spinosa*, *Tara tinctoria*.

I. INTRODUCTION

History:

Obtained by grinding the endosperm of the seeds of Caesalpinia spinosa (Fam. Leguminosae); consists chiefly of polysaccharides of high molecular weight composed mainly of galactomannans. The principal component consists of a linear chain of (1,4)-beta-D mannopyranose units with alpha-D-galacto- pyranose units attached by (1 6) linkages; the ratio of mannose to galactose in tara gum is 3:1. (In carob bean gum this ratio is 4:1 and in guar gum 2:1.) The article of commerce may be further specified as to viscosity and loss on drying.

Coyote Brand Tara Gum, Caesalpinia Spinosa, is part of the Leguminosae family. Tara is mainly produced in Peru from April through December.

Regulatory:

Tara Gum is a naturally high molecular weight hydrocolloid polysaccharide. It is composed of galactan arrd mannan units combined through glycosidic linkages. It may be described chemically as a galactomannary like guar and locust bean gum. ln the US, it is given GRAS status by the FDA. Tara Gum fully meets all specifications as outlined in the Food Chemicals Codex and may be used safely in food as described in the Federal Register, in additon to pharmaceutical purposes as described in the US Pharmacopeia.

DESCRIPTION	White to white-yellow, nearly odourless powder
FUNCTIONAL USES	Thickening agent, stabilizer

Scientific classification	
Kingdom:	Plantae
(unranked):	Angiosperms
(unranked):	Eudicots
(unranked):	Rosids
Order:	Fabales
Family:	Fabaceae
Genus:	*Caesalpinia*
Species:	***C. spinosa***
Binomial name	
Caesalpinia spinosa	

CHARACTERISTICS

IDENTIFICATION

Solubility (Vol. 4) Soluble in water; insoluble in ethanol. wate
Gel test To an aqueous solution of the sample add small amounts of sodium borate; a gel is formed.
Viscosity Transfer 2 g of the sample into a 400-ml beaker and moisten it thoroughly with about 4 ml of isopropanol. Add, with vigorous stirring, 200 ml of water and continue stirring until the gum is completely and uniformly dispersed. An opalescent, moderately viscous solution is formed. (This solution is less viscous than a guar gum solution, but more viscous than a carob bean gum solution when prepared and tested as indicated in the above described test). Transfer 100 ml of this solution into another 400-ml beaker, heat the mixture in a boiling water-bath for about 10 min and cool to room temperature. The solution shows a marked increase in viscosity.
Gum constituents Proceed as directed under Gum Constituents Identification, using galactose and mannose as standards. Galactose and mannose should be present
Microscopic examination Place some ground sample in an aqueous solution containing 0.5% iodine and 1% potassium iodide on a glass slide and examine under a microscope. Tara gum contains groups of round to pear-shaped cells; their contents are yellow to brown.
(Guar gum cells are similar in form but markedly larger in size. Carob bean gum shows long, stretched tubiform cells, separate or slightly interspaced and can be easily distinguished from tara gum.

PURITY

Loss on drying Not more than 15%
Ash Not more than 1.5%
Acid insoluble matter Not more than 2%
Protein Not more than 3.5%
Proceed as directed under Nitrogen Determination (Kjeldahl method) .The percentage of nitrogen determined multiplied by 5.7 gives the percentage of protein in the sample.
Starch Not detectable To a 1 in 10 solution of the sample, add a few drops of iodine TS. No blue colour is produced.
Lead Not more than 2 mg/kg Determine using an atomic absorption technique appropriate to the specified level. The selection of sample size and method of sample preparation may be based on the principles of the method described in Volume 4, "Instrumental Methods."

Fig.1 Tara tree

Fig.2 Tara tree

Fig.3 Tara Gum

Fig.4 Tara gum

Fig.5 Tara seed

Fig.6 Tara Gum

Fig.7 Tara powder

Functional Properties:

Tara Gum is unique in that it bridges the gap between the cold soluble, highly galactose substituted guar and cold insoluble, lower galactose substituted Locust Bean Gum. Tara Gum shows associative or synergistic effects similar to Locust Bean Gum, i.e. enhanced viscosity and gelation with certain other hydrocolloids including Kappa and Iota Carrageenans and Xanthan Gum. Similar to Locust Bean Gum, Tara Gum solutions are highly viscous with a short texture. Tara Gum in frozen dessert provides fat-like texture, smooth eating properties and excellent heat shock protection. The mixture of Tara arrd Xar-rthanG ums provides excellent long term suspensions, suggesting its potential in salad dressings, sauces and similar products.

Food Applications:

Tara Gum provides food processors with many advantages in a wide range of non-fat and low-fat food applications including: frozen deserts, cultured dairy products, condiments, baked goods and salad dressings. Like guar, Tara Gum is cold-soluble and attains maximum viscosity in water, milk and other low-solid systems within several minutes. Similar to Locust Bean Gum, Tara Gum acts synergistically with Kappa Carrageenan and Xanthan Gum to increase gel strength arrd make such gels less Drone to svneresls. Tara Gum provides cold-water solubility and viscosity at 25'C. Unlike Locust Bean Gum, Tara Gum provides outstanding heat shock protection, impats a rich buttery mouthfeel, requires usage levels of 20 to 25 percent less than Locust Bean Gum and resists breakdown during high shear.l n comparisont o Locust BeanG um, TaraG um gives a better texture in frozen dessertsw ithout gumminessa nd is functional at lower usage levels.

Methods

Plant material

C.spinosa pods were collected in Villa de Leyva, Boyacá, Colombia in March 2007 and identified by Luis Carlos Jiménez from the Colombian National Herbarium; voucher specimen number COL 523714.

Plant extraction and purification

Three kg of fresh pods from C.spinosa were dried under airflow in a solar oven at 35°C and ground down to obtain 1.8 kg of plant material. Subsequently the plant

material was extracted with ethanol (96%, 10 L) in a recirculating percolator (twice per day) over a period of 10 days. The ethanol crude extract (80 g) was concentrated under vacuum, trapped on silica gel and excess humidity removed at 25°C. Afterwards, the ethanol extract was fractionated with the following solvents: petroleum ether (1.5 L); chloroform (2 L); ethyl acetate (2 L); ethanol (2 L) and water (2 L) (aqueous fraction). From the ethyl acetate fraction we obtained an abundant precipitate which we named (P2Et). This corresponded to 2.78% of the ethanol extract and a supernatant which we named (S2Et) corresponded to 1.11%. The P2Et, S2Et and aqueous fractions were selected for biological testing based on their cytotoxic activity. The extraction protocol was performed three times and the chromatographic profiles of the components were verified. The quality control carried out on the P2Et fraction gave the following results: foreign matter less than 2%; total ash less than 8%; ash that was insoluble in hydrochloric acid less than 1%; no evidence of heavy metals and pesticides. These result met the British Herbal Pharmacopoeia

quality parameters.

Phytochemical characterization

Fraction characterization was determined by means of standard phytochemical tests. In the total ethanol extract the presence of alkaloids or nitrogen compounds were not identified using Dragenddorff, Valser, Reineckate and Mayer’s reagents. The Shinoda test (Mg in HCl) was positive suggesting the presence of flavanones, flavanonols,

flavones, flavonols or isoflavones. Hydrogen peroxide evidenced the presence of naphthoquinones and/ or anthraquinones. The presence of steroids was demonstrated using Liebermann Burchard reagent. Low concentrations of steroidal saponins and/or triterpenoids were detected using hemolysis and foam tests. In order to assess the presence of anthraquinone glycosides, Borntrager's reaction (treatment with ammonia solution) was used. The presence of tannins was verified using ferric chloride solution, gelatin and lead acetate.. P2Et, S2Et and aqueous fractions exhibited the presence of leucoanthocyanidins, the absence of quinones and a significant tannin content, especially in P2Et fraction.

Thin layer chromatography (TLC)

Chromatographic analysis was carried out on TLC aluminium sheets (10 × 5 cm) (Merck) silica gel 60 F 254. Three solvent systems were used: Petroleum ether - thyl acetate - formic acid (40:60:1); chloroform – ethyl acetate - acetic acid (50:50:1); and toluene – acetonitrile - formic acid (70:30:1). After basic hydrolysis, the P2Et and S2Et fractions were dissolved in methanol (1%) and detected using UV (254 nm), FeCl3 (10%) and vanillinsulfuric acid (VS)/110°C. Gallic acid was used as a positive control.

HPLC - PDA-MS

HPLC analysis was carried out in an Alliance 2795 (Waters®, UK) with a PDA detector (996). A Sunfire (Waters) column C18 - 2.1 × 150 mm × 5 µm was used, with a flow rate of 0.25 ml/min and a linear gradient from 95% solvent A (H2O + 1%CH3COOH) and 5% solvent B (CH3CN) to 60% in solvent A and 40% in solvent B, over a period of 25 min. The mass spectrum (MS) analysis was carried out using a LCT paper guidelines, please contact the conference publications committee as indicated on the conference website. Information about final paper submission is available from the conference website. mass spectrometer with an ESI source. The percentage relative abundance was determined using quercetin as an internal standard (0.0625 µg/µl). Runs were performed in triplicate. Tumor cell line and normal cells The cell lines used as cancer cells were K562, a human erythroleukemia and MCF7, a human breast adenocarcinoma, from the American Type Culture Collection (ATCC); and the cell lines used as normal cells were human peripheral blood mononuclear cells (PBMC) and human fibroblasts obtained from normal healthy donors after informed consent was given. This project was approved by the ethics committee (founded in 2002) of the Science Faculty at a meeting on August 21, 2007. The culture conditions under which the cell lines were maintained have already been reported . In vitro cytotoxicity assaysThe cytotoxic effects of the fractions and conventional drugs (doxorubicin, etoposide, vincristine and taxol) were evaluated using normal and tumor cells by means of trypan blue and the methylthiazol tetrazolium (MTT) assay, as previously reported . The P2Et fraction was dissolve in ethanol and the corresponding vehicle was used as a negative control. Measurement of mitochondrial membrane potential The cells were treated with different concentrations of the P2Et fraction or valinomycin (positive control, 0.1 µg/ml) for 4, 8 and 12 h for K562 cells, and for 6, 12and 24 h for MCF7 cells. The mitochondrial membranepotential (MMP) was measured using JC-1 dye, as previously described.

Annexin V assay

Phosphatidylserine (PS) externalization was assessed by flow cytometry using Annexin V-FITC (Molecular Probes, Invitrogen Corp, Carlsbad, CA, USA)/PI (Sigma, Saint Louis, MO, USA). K562 and MCF7 cells (3 × 105) were treated with doxorubicin, ethanol or the P2Et fraction for 48 h. After treatment, cells were suspended in Annexin buffer (Hepes 100 mM, NaCl 140 mM, CaCl2 2.5 mM) and incubated with Annexin V-FITC for 8 min at room temperature. Then the cells were incubated with PI for 2 min at 4°C, acquired on a FACSAria I (Becton Dickinson, New Jersey, USA) and analyzed with FlowJo software (Tree Star Inc., Ashland, USA). Results are expressed as the mean ± SE of three independent experiments. Caspase 3 assays

Caspase 3 activity was estimated using the caspase 3 colorimetric assay kit, which detects enzyme activity based on the cleavage of Asp-Glu-Val-Asp (DEVD)-pNA (R&D Systems Inc., Minneapolis, MN, USA). Briefly, cells (2 × 105 cells/ml) were cultured using different concentrations of the P2Et fraction and doxorubicin (positive control) or ethanol (negative control) for 48 h. After the cells were ice lysed for 10 min the enzyme activity was measure on 96-well flat-bottom microplates with 50 µl of supernatant. The supernatant was prepared by centrifuging at 10,000 × g for 1 min (100-200 µg of total protein), and then adding 50 µl of reaction buffer supplemented with 10 µl of DTT and 5 µl of caspase 3 colorimetric substrate DEVD-pNA. Next cells were incubated for 1 ± 2 h at 37°C and caspase-3 activity was measured at 405 nm on a spectrophotometer (Multiskan Labsystem). The increase in caspase 3 activity was calculated relative to the absorbance value of the negative control DNA fragmentation and cell cycle analysis DAPI (4',6-diamidino-2-phenylindole, Sigma) stained cells were monitored under a microscope as previously described. Slides were mounted using prolong antifade kit (Molecular Probes, Eugene, Oregon, USA) and cells were analyzed under a fluorescence microscope (Olympus, Japan). Cell cycle analysis was undertaken aspreviously reported .

Clonogenic assays

The clonogenic assays were performed as previously described . Briefly, K562 human cells (2.5 × 105 cells/well) were plated (96-well plate) and treated with the P2Et fraction at 40 and 20 µg/ml, or with 15 and 6 µg/ml etoposide, or 0.2% ethanol (in PBS) and incubated for 24 h under a humidified environment at 37°C and 5% CO2. After treatment cells were re-plated onto 0.5% agar dishes (60 mm, 20,000 cells/dish), incubated for 14 days (37°C and 5% CO2) and stained with violet crystal (0.4% in ethanol). Cell colonies with more than 50 cells were counted. Treatments were performed in triplicate, and results expressed as mean ± SE. P2Et fraction adjuvant activity P2Et fraction adjuvant activity was assessed using K562 and MCF7 cells in combination with the well-known cancer treatment drugs doxorubicin, vincristine, taxol and camptothecin. Cell viability was evaluated by means

of the MTT assay. K562 and MCF7 cells (5 × 103 cells/ well) were seeded in 96-well plates and treated for 6 h with sublethal concentrations of the P2Et fraction (1.6 μg/ml, 27 fold less than the IC50 value for K562 cells and 15.5 μg/ml for MCF7 cells); washed and incubated in fresh medium with each drug for 48 h at 37°C in humid atmosphere and 5% CO2. Sublethal concentrations of the chemotherapeutic drugs had been previously determined by MTT assay. Results are expressed as cell viability percentage relative to the control (100 × Treatment OD/Negative control OD).

Statistical analysis

Data is presented as the mean ± SE. The data were analysed by one- and two-way ANOVA and differences between control and treated groups were determined using the Bonferroni and Tukey tests. Differences were considered significant for $p < 0.05$ and were determined using the GraphPad prism 5.0 software.

APPLICATIONS

C. spinosa pods are an excellent source of environmentally friendly tannins (*tara tannins*) most commonly used in the manufacture of automotive and furniture leathers. This growing industry is developing around their production in Peru. Some producers have their own plantations to guarantee constant quality. Tara tannin derivatives are being proposed as antifouling against marine organisms that can grow on ship hulls. Those tannins are of the hydrolysable type. Gallic acid is the main constituent of tara tannins (53%) and can be easily isolated by alkaline hydrolysis of the plant extract. Quinic acid is also a constituent of the tara tannins.

Tara gum is a white or beige, nearly odorless powder that is produced by separating and grinding the endosperm of *C. spinosa* seeds. Tara gum consists of a linear main chain of (1-4)-ß-D-mannopyranose units attached by (1-6) linkages with α-D-galactopyranose units. The major component of the gum is a galactomannan polymer similar to the main components of guar and locust bean gums that are used widely in the food industry. The ratio of mannose to galactose in Tara gum is 3:1.[13] Tara gum has been deemed safe for human consumption as a food additive. Tara gum is used as a thickening agent and stabilizer in a number of food applications. A solution of tara gum is less viscous than a guar gum solution of the same concentration, but more viscous than a solution of locust bean gum. Generally tara gum presents a viscosity of around 5,500 cps (1% aqueous solution). Furthermore, tara gum shows an intermediate acid stability between locust bean gum and guar gum. It resists the depolymerisation effect of organic acids down to a pH of 3.5. This gum is also stable to high temperature heat treatment, up to 145°C in a continuous process plant. Blends of tara with modified and unmodified starches can be produced which have enhanced stabilization and emulsification properties, and these are used in the preparation of convenience foods, such as ice cream.

Medicinal uses in Peru include gargling infusions of the pods for inflamed tonsils or washing wounds; it is also used for fevers, colds and stomach aches. The tree can also be a source of lumber and firewood, and as a live fence. Water from boiled dried pods is also used to kill fleas and other insects. The seeds can be used to produce black dye while dark blue dye can be obtained from the roots.

CONCLUSION

Tara Gum is a naturally high molecular weight hydrocolloid polysaccharide. It is composed of galactan arrd mannan units combined through glycosidic linkages. It may be described chemically as a galactomannary like guar and locust bean gum. ln the US, it is given GRAS status by the FDA. Tara Gum fully meets all specifications as outlined in the Food Chemicals Codex and may be used safely in food as described in the Federal Register, in additon to pharmaceutical purposes as described in the US Pharmacopeia.

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