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RESEARCH ARTICLE

Kiranmai Mudunuri et al, The Experiment, 2019, Vol 47 (2), 2657-2667

Neuromodulatory activity of *Calophyllum Antillanum* root water extract

Abstract: Calophyllumantillanum is an evergreen, medium-sized tropical tree in the Calophyllaceaefamily. It is also known as Antilles calophyllum; Alexandrian laurel; Galba; Santa Maria; mast wood, beauty leaf, West Indian laurel. Neuromodulator activity was tested with CalophyllumAntillanum root water extract. In this study CalophyllumAntillanum root water extract clearly impacts on Hormone releasing up to day-3. Amylin, Gastrin, Calcitonin, Cortisosterone, Cortisol harmones percentage are clearly increasing in blood day-1 to day-3. According to research report the CalophyllumAntillanum primary metabolites, secondary metabolites showing impact on Pancreas, Kidneys, Heart, Adrenal glands, Gonads, Thyroid, Parathyroid, Thymus. Its causes increase of hormones levels in blood. The CalophyllumAntillanum is very useful to cure so many Neuro problems.
Key words: CalophyllumAntillanum, Neuromodulator activity, Phytochemical screening.

Introduction:

Calophyllum antillanumis an evergreen, medium-sized tropical tree in the Calophyllaceae family. It is also known as Antilles calophyllum; Alexandrian laurel; Galba; Santa Maria; mast wood, beauty leaf, West Indian laurel. It is prized for producing a very hard, durable wood. "The leaves were once used as a diuretic in Grenada, but it is said in Dominica to be poisonous. Famous hard wood . Very long lasting hut construction."[1] It is considered an invasive weed species in some areas.[2] "The wood of maría is widely used in the tropics. The heartwood varies from yellowish pink through reddish brown while sapwood is generally lighter in color. The grain is usually interlocked, and the specific gravity ranges from 0.51 to 0.57. The wood is fairly easy to work, rating above average in shaping, sanding, and mortising, and below average in planning, turning, and boring. It is moderately difficult to air-season and shows moderate to severe warp. The sapwood is easily impregnated with preservatives by either pressure or open-tank-bath methods, but the heartwood is extremely resistant to impregnation."[3]. María wood is suitable for general construction, flooring, bridge construction, furniture, boat construction, cabinetmaking, shingles, interior construction, agricultural implements, poles, crossties, and handles. It is a good general utility wood where a fairly strong and moderately durable timber is required. In British Honduras, it was substituted for imported creosoted sleepers but required replacement after 3 or 4 years. In Mexico, attempts to use the timber in the veneer and plywood industry were not entirely successful. The tree is also planted for shade along streets and as a windbreak or to protect against salt spray near the ocean. Frequently it is pruned to form a dense hedge along property lines in urban areas.

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Image.1 Leaf and flower of Calophyllum Antillanum

The latex from the trunk has been employed medicinally. The fruits are used as hog-feed, and lamp oil is extracted from the seeds. The tree's adaptability to a variety of sites in Puerto Rico has made it popular among soil scientists and foresters for rehabilitation of degraded lands."[3] It is native to the Caribbean region, including Antigua and Barbuda; Barbados; Cuba; Dominica; Grenada; Guadeloupe; Hispaniola; Jamaica; Martinique; Montserrat; Puerto Rico; St. Lucia; St. Vincent and the Grenadines; Trinidad and Tobago; Virgin Islands (U.S.) - St. Croix. It has also been introduced to Florida and Hawaii[4][5] In Trinidad it was used to make spinning tops for children.[6] It has also been reported from Costa Rica, Colombia, Mexico, El Salvador, Puerto Rico, Ecuador, and Paraguay, etc.[7] Galba, its common name in Trinidad, may have been the origin of the stage name of Grenadian-born calypsonian, Sir Galba.[6]

Medicinal Uses:

Plants for a Future cannot take any responsibility for any adverse effects from the use of plants. Always seek advice from a professional before using a plant medicinally. The resin obtained from the crushed or cut bark, called balsmo de mara, has been used medicinally. A decoction of the trunk bark, combined with the root-bark of Coutareahexandra, is used as an antidiabetic and vermifuge. The plant (part not specified) is used to dress sores, and as a headache remedy. The plant contains xanthones, including guanandine, isoguanandine and jacareubine.

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Materials and Methods:

The list of equipment with make are showed in Table.1. All reagents are analytical grade. Required animals are purchased from RaajFarms, Shastri Nagar, Chennai, India.

S.No	Equipment	Make
1	Electronic balance	Denver
2	Spectrophotometer	TECH CHOMP
3	HPLC	Peak LC P7000 version 1.06 (Isocratc)
4	HPLC detector	UV- VIS detector(UV7000)
5	HPLC Column	Intersil ODS C18 column (250 mm × 4.6 mm×5µ)

Table.1 List of Equipment

Preparation of plant root extract:

The collected Calophyllum Antillanum roots were dried under shade, powdered and sieved through 40mesh. About 100g of powdered materials were extracted with petroleum ether (60°-80°C) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is dissolved in tween 80 and used for the experiment.

Preliminary phytochemical screening:

1. Test forsteroids:

Salkowski Test: Few drops of concentrated sulphuric acid are added to the plant extract, shaken and on standing; lower layer turns red in colour.

Liebermann Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a reddish brown ring is formed at the junction of two layers.

2. Tests fortriterpenoids:

Salkowski Test: Few drops of concentrated sulphuric acid is added to the extract, shaken and on standing, lower part turns golden yellow color.

Lieberman Burchard's Test: To the extract, few drops of acetic anhydride is added and mixed well. 1 ml of concentrated sulphuric acid is added from the sides of test tube, a red ring indicates triterpenes.

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Ischugajiu Test: Excess of acetyl chloride and pinch of zinc chloride are added to the extract solution, Kept aside for reaction to subside and warmed on water bath, cosin red colour is produced.

Brickorn and Brinar Test: To the extract, few drops of chlorosulfonic acid in glacial acetic acid (7:3) are added, red colour is produced.

3. Test forSaponins:

Foam Test: Small amount of extract is shaken with little quantity of water, the foam produced persists for 10 minutes. It confirms the presence ofsaponins.

HaemolysisTest: To 2ml of 1.8% Sodium chloride solution in two test tubes, 2ml distilled water is added to one and 2ml of 1% extract to the other, 5 drops of blood is added to each tube and gently mixed with the contents. Haemolysis observed under the microscope in the tube containing the extract indicates the presence ofsaponins

4. Test for SteroidalSaponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer istested for steroids.

5. Tests for Triterpenoidal Saponin:

The extract is hydrolysed with sulphuric acid and extracted with chloroform. The chloroform layer istested for triterpenoids.

6. Tests forAlkaloids:

Mayer's Test: The acid layer when mixed with Mayer's reagent (Potassium mercuric iodide solution) gives creamy white precipitate.

Dragendroff's Test: The acid layer with few drops of Dragendroff'sreagent (Potassium bismuth iodide) gives reddish brown precipitate.

Wagner's Test: The acid layer when mixed with few drops of Wagner's reagent (solution of iodide in potassium iodide) gives brown to red precipitate.

Hager's Test: The acid layer when mixed with few drops of Hager's reagent (Saturated solution of pricric acid) gives yellow colouredprecipitate.

7 Tests for Carbohydrates:

Fehlings's Test: The extract when heated with Fehling's A and B solutions gives an orange red precipitate showing the presence of reducing sugar.

Molischs's Test: The extract is treated with Molisch's reagent and conc. sulphuric acid along the sides of the test tube, a reddish violet ring shows the presence of carbohydrate.

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Benedict's test: The extract on heating with Benedict's reagent, brown precipitate indicates the presence of sugar.

Barfoed's Test: Barfoed'sreagent is added and boiled on water bath for few minutes, reddish precipitate is observed for the presence ofcarbohydrate.

8. Test forFlavonoids:

Shinoda Test: The extract solution with few fragments of magnesium ribbon and concentrated hydrochloric acid produced magenta colour after few minutes.

Ferric chloride test: Alcoholic solution of extract reacts with freshly prepared ferric chloride solution and given blackfish green color.

Lead Acetate Test: Alcoholic solution of extract reacts with 10% lead acetate solution and given yellow precipitate.

9. Test forGlycosides:

Anthraquinone test: Drug is powdered and extracted with either ammonia or caustic soda. The aqueous layer shows pink color

Keller-killiani test: This is for cardiac glycosides. The extract and 0.4 glacial acetic acid are mixed with ferrous chloride and 0.5 mi of concentratedsulphuric acid. The acetic acid layer shows blue color

10. Test for PhenolicCompounds:-

Ferric chloride test:-Treat the extract with ferric chloride solution then blue color appears if hydrolysable tannins are present and green color appears if condensed tannins are present.

Gelatin test: - To the test solution add 1% gelatin solution containing 10% Nacl, and then ppt is formed.**Test for chlorogenic acid:-**Treat the test solution with aqueous ammonia and expose to air gradually, green colour is developed.

Neuromodulatory activity of Calophyllum Antillanum root extract:

Acute toxicity study:

Animals were observed for four hours hourly for behavior changes and daily for six days. The extract was devoid of any toxicity in rats when given in dose up to 100 mg/kg by oral route. Hence, for further studies 100-500 mg/kg doses of extract were used. Finally 500 mg/kg dose was fixed for experiment.

Experimental Design for Neuromodulator activity:

The 30rats were divided in to five groups (n =6 animals). Drugs/ vehicle were administered to the animals 60min prior to study.

Group A: Standard drug Donepezil (5 mg/kg orally).

Group B: Control, administer saline (5 ml/kgorally).

Group C: Water Extract with 100mg/kg

Group D: Water Extract with 250mg/kg

Group E: Water Extract with 500mg/kg

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A Neuromodulator is a messenger released from a neuron in the central nervous system, or in the periphery, that affects groups of neurons, or effector cells that have the appropriate receptors. It often acts through second messengers and can produce long-lasting effects. In addition to the nervous system, the endocrine system is a major communication system of the body. While the nervous system uses neurotransmitters as its chemical signals, the endocrine system uses hormones. In this study Amylin, Gastrin, Calcitonin, Cortisosterone, Cortisolhormones are quantified before and after the treatment with Calophyllum Antillanum roots water extract, and compared impact of root extract with Standard drug.Hormones are analyzed& quantified with HPLC. HPLC method for quantification of Amylin, Gastrin, Calcitonin, Cortisosterone,Cortisolhormones:

Sample preparation [12]:

Rat plasma stabilized with K_2EDTA (Lampire Biological Laboratories, Pipersville PA) was spiked with the analytes from a standard solution. A 100 µL aliquot was added to the Hybrid SPE-Phospholipid plate followed by 300 µL of 1% formic acid acetonitrile precipitation solvent. The plate was agitated via vortex for 4 minutes, placed on vacuum manifold and subjected to 10" Hg vacuum for 4 minutes. The filtrate was collected and analyzed directly. The concentration of steroid hormones in the final sample work up was equivalent to 50 ng/mL. Recovery was based on interpolation of a standard curve of analytes in buffer.

HPLC Conditions:

S.No	Condition	Parameter
1	Mobile Phase	5 mM ammonium formate, pH 4.0 with formic acid; (B) methanol; **gradient:** 60% B for 30 min, to 95% B in 5 min, held at95% B for 30 min;
2	Column	C18, 250mm
3	Pump	Gradient
4	Sample volume	2.0µL
5	Detector wavelength	266 nm
6	Flow rate	0.3 mL/min
7	**column temp**	50 °C

Table.2 HPLC Conditions for the quantification of Neuromodulatorhormones.

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Results and Discussion

The amount of Neuromodulator hormones are quantified at Zero hour and after 12 hours of treatment. The HPLC analysis run up to 60 min. Cortisosterone eluted at 7.45 min, Amylin eluted at 14.8 min, Calcitonin eluted at 30 min, Cortisol eluted at 42.5 min, Gastrin eluted at 46.05 min. The results are showedin Table.3 and Table.4

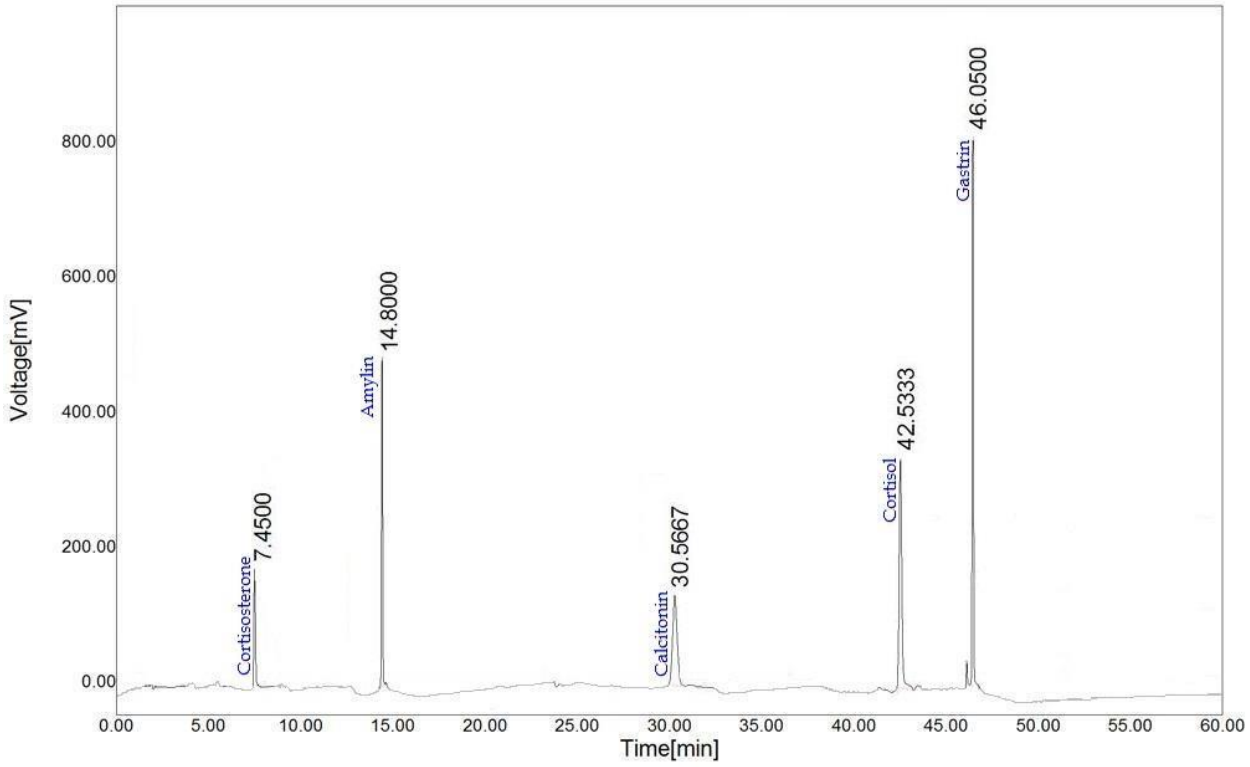

Figure.3 Standard HPLC chromatogram of Amylin, Gastrin, Calcitonin, Cortisosterone, Cortisol

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Group and Drug	**Amylin (mg/dl)**	**Gastrin (mg/dl)**	**Calcitonin (mg/dl)**	**Cortisosterone (mg/dl)**	**Cortisol (mg/dl)**
Group A: Standard drug Donepezil (5 mg/kg orally).	4.6±0.48	10.4±0.39	5.9±0.63	14.6 ±0.40	9.6±0.08
Group B: Control, administer saline (5 ml/kg orally).	5.5±0.35	9.6±0.54	6.6±0.85	13.5±0.31	10.2±0.63
Group C: Water Extract with 100 mg/kg	6.2±0.02	10.9±0.62	6.05±0.76	14.2±0.69	11.4±0.47
Group D: Water Extract with 250 mg/kg	5.8±0.76	9.2±0.74	6.3±1.32	15.3±0.92	9.4±0.53
Group E: Water Extract with 500 mg/kg	4.9±1.20	8.9±0.93	5.6±0.42	13.9±1.53	11.6±0.82

Table.3 Quantification of Neuromodulator hormones at zerohour.

Group and Drug	**Amylin(mg/dl)**	**Gastrin (mg/dl)**	**Calcitonin (mg/dl)**	**Cortisosterone (mg/dl)**	**Cortisol (mg/dl)**
Group A: Standard drug Donepezil (5 mg/kg orally).	15.5±0.16	21.4±0.95	14.6±0.03	24.9±0.84	20.4±1.63
Group B: Control, administer saline (5 ml/kg orally).	5.3 ±0.48	11.5 ±0.39	6.2 ±0.63	14.6 ±0.40	10.5±0.08
Group C: Water Extract with 100 mg/kg	9.2 ±0.52	12.5 ±0.47	8.3 ±1.24	16.8 ±1.35	13.2±1.26
Group D: Water Extract with 250 mg/kg	11.5 ±0.63	16.8 ±0.59	10.2 ±1.52	20.5±1.29	16.8±0.63
Group E: Water Extract with 500 mg/kg	14.6 ±0.05	20.6 ±1.21	13.5±0.26	22.9±0.52	19.5±0.43

Table.4 Quantification of Neuromodulator hormones at 12thhour.

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Neuromodulation is the process by which nervous activity is regulated by way of controlling the physiological levels of several classes of neurotransmitters. Neuromodulators are a subset of neurotransmitter. Unlike neurotransmitters, the release of neuromodulators occurs in a diffuse manner. This means that an entire neural tissue may be subject to the neuromodulator's action due to exposure. This, in turn, can tune the neural circuitry of an entire brain region; not just that of an individual neuron. This is in contrast with the release of a neurotransmitter, which occurs at a specific synapse during direct synaptic transmission. Furthermore, neuromodulators and neurotransmitters act on different types of neuroreceptors.While neurotransmitters target fast-acting "ionic" neuroreceptors that convey electrochemical signals into the target neuron, neuromodulators target the slower G-protein neuroreceptors.

Importantly, the act of neuromodulation, unlike that of neurotransmission, does not necessarily carry excitation of inhibition from one neuron to another, but instead alters either the cellular or synaptic properties of certain neurons so that neurotransmission between them is changed. ympathomimetic and sympatholytic drugs: these enhance and block at least some of the effects of noradrenaline released by the sympathetic nervous system, respectively. Dopamine reuptake inhibitors: these prevent dopamine reuptake by blocking the action of the dopamine transporter. These drugs are frequently used in the treatment of conditions including ADHD, depression and narcolepsy. Selective serotonin reuptake inhibitors: these temporarily prevent the removal of serotonin from specific synapses, thereby enhancing the effect of released serotonin. These are used in the treating depression. Cholinesterase inhibitors: these bind to cholinesterase resulting in increased acetylcholine in the synapses. These are used to treat dementia in patients with Alzheimer's disease. Neuromodulation is also a category of treatment that involves stimulation or direct administration of medications to the body's nervous system for therapeutic purposes. This aims to modulate activity of target cells as an approach to pain control and neurological dysfunction by treating movement disorders, conditions such as spasticity and epilepsy, as well as pain syndromes. In this present study, there is no change in Neuromodulators release in control group animals (Group-B). There is gradual increase in the concentration of Neuromodulators in group C, Group-D, Group-E. The standard drug treated animals (Group-A) and High dosages water extract treated animal groups (Group-E) are showed similarresults.

Conclusion:

A neuromodulator is a messenger released from a neuron in the central nervous system, or in the periphery, that affects groups of neurons, or effect or cells that have the appropriate receptors. it often acts through second messengers and can produce long-lasting effects. In addition to the nervous system, the endocrine system is a major communication system of the body. While the nervous system uses neurotransmitters as its chemical signals, the endocrine system uses hormones. In this study

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