EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 12
157-171
Year: 2022

A COMPREHENSIVE DOCKING STUDY REVIEW OF PHARMACOGNOSTIC, PHYTOCHEMICAL AND PHARMACOLOGICAL STUDIES OF ACACIA AURICULOFORMIS A.CUNN.EX BENTH. WITH IMMUNOMODULATORY ACTIVITY.

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Article Received on 09/10/2022 **Article Revised on 29/10/2022** **Article Accepted on 19/11/2022**

ABSTRACT
Immunomodulatory treatment is often required beneath the conditions of impeded safe responsiveness and when the resistant components have got to be actuated. Even while normal immunomodulatory chemotherapy is available, it is so expensive that it is frequently out of reach of average socioeconomic people. As a result, the balancing of safe framework by conventionally used medicinal plant products has become a topic for recent logical studies all over the world. The perennial shrub Acacia auriculiformis A.Cunn. ex Benth. is widely dispersed throughout the world and has a variety of therapeutic potentials. It has been used traditionally to treat a variety of medical issues, including allergies, rheumatism, sore eyes, pains, and rashes. Due to its low toxicity (LD_{50} = 3741.7 mg/kg) and high efficacy, Acacia auriculiformis has also been shown to have numerous pharmacological effects, including central nervous system depressant activity, antioxidant, antimicrobial, antimalarial, anti-filarial, cestocidal, antimutagenic, chemopreventive, spermicidal, wound healing, hepatoprotective, and antidiabetic. The presence of the main components- flavonoids (Auriculoside) and triterpenoid saponin glycosides (acaciasides- acaciaside A & B) in various portions of this plant is also demonstrated by numerous phytochemical analysis. Researchers have been doing several investigations on this medicinally significant plant for many years in an effort to elicit the diverse biological actions. Significant effectiveness was shown in the numerous plant extracts tested for diverse pharmacological actions. The plant's bioactive phytoconstituents that have been isolated from various plant sections are emphasised for different pharmacological activity. It is also said that the plant underwent pharmacognostical standardisation using multiple standards characteristics. This plant's low toxicity and the presence of important bioactive phytoconstituents including flavonoids and triterpenoid, saponin glycosides are what make it useful as a drug treatment for numerous illnesses. In-depth, details regarding the pharmacognostic, phytochemical, and pharmacological studies of Acacia auriculiformis to date as well as the plant's immunomodulatory properties are included in this article.

KEYWORDS: Immunomodulatory, Acacia auriculoformis, antimicrobial, antioxidant, cestocidal, Antifungal, Antifilarial.

1. INTRODUCTION
The development of human history demonstrates that traditional medicine is used for therapeutic purposes. According to the World Health Organization (WHO), because to poor or nonexistent access to medical care, 70% to 80% of the population relies mostly on animal and plant-based remedies. In addition to being employed as traditional remedies, substances derived from wild plants and animals are also used as raw materials in the creation of contemporary allopathic and herbal therapies.[1]

A disease is treated using immunotherapy, also known as immunomodulatory activity, by inducing, advancing, or overcoming a resistant response. Immunostimulants are defined as immunotherapies that are designed to induce or intensify a safe response. Conversely, immunotherapies designed to lessen or suppress are referred to as immunosuppressants.[52]

Immunotherapies based on cells have proven effective in treating various malignancies. Natural killer cells (NKs), cytotoxic T lymphocytes (CTL), dendritic cells,

lymphocytes, macrophages, and other immune effector cells work in concert to protect the body against cancer by identifying unique antigens present on the surface of the malignant cells as a result of mutation.[53]

To understand the appealing effects on infection avoidance, the immunomodulating properties of plants are being examined comprehensively. Therefore, home remedies have been used for generations due to their security, sufficiency, few adverse effects, and social acceptability. Plants and their derivatives are therefore harmless, and as a result, patients are continuously treated with plant products as a choice, a practice that dates back to ancient Poland.[54]

By stimulating or suppressing the formation of serum antibodies, immunomodulatory medicines alter how the immune system reacts to an antigen. Immunostimulators are given to improve the immune response to, among other things, tumours, primary or secondary immunodeficiency, infectious illnesses, and changes in antibody transfer. Drugs that inhibit the immune system are used to treat autoimmune conditions such lupus, allergies, and pemphigus as well as transplanted organ rejection.[55,56]

The oldest and most popular type of medication is derived from medicinal plants.[1] It is unclear when people first learned how to employ medical plants and other natural compounds.[2] Plants were first used as medicines in the form of unrefined substances like tinctures, teas, poultices, powders, and other herbal preparations. The isolation of active chemicals has been a recent step in the use of plants as medicines.[3] One such priceless species is Acacia auriculiformis A. Cunn. ex. Benth, from which several therapeutically effective components have been extracted. It is a leguminous tree with several uses that is significant for forestry and medicine.[4]

The Fabaceae family tree Acacia auriculiformis (A. auriculiformis) A.Cunn. ex Benth. is a straight, medium-sized, deciduous or evergreen tree that may grow as tall as 30 meters and is typically seen in parks and along Indian highways. The Greek word "akis," which indicates a spike or a point, is where the general name of the acacia tree comes from. While the Latin term "forma" denotes a frame, figure, or shape, the English word "auricula" refers to a creature's external ear. The tree was initially brought to India in 1946 in West Bengal and is a native of Australia.[2] The tree is abundant in galactose, arabinose, rhamnose, glucuronic acid, and methylglucuronic acid.[3] Various pharmacological effects of the plant have been described, including antioxidant[4], antimicrobial[5], antimalarial[6], antifilarial[7], cestocidal[8], antimutagenic[9], spermicidal[10], hepatoprotective[11], wound healing[12], and antidiabetic action.[11] The flavone glycosides auriculoside, which was isolated from A. auriculiformis, was found to have central nervous system (CNS) depressive action.[9,13,14]

This species is grown for decorative and shade reasons in urban areas where its hardiness, thick foliage, and vibrant yellow blooms are assets. Acacia auriculiformis A. Cunn. ex Benth was given the botanical name in Hooker's London J. Bot. 1: 377. (1842). Because of the shape of the legume, the specific name is a play on the Latin words auricular, which refers to an animal's external ear, and forma, which means form, figure, or shape. The plant's common trade name in Australia is northern black wattle, although it also goes by the names ear-pod wattle, Darwin black wattle, and tan wattle.

2. TAXONOMIC AND BOTANICAL DESCRIPTION

The specific name of A. auriculiformis is A. auriculiformis A.Cunn. ex Benth. comes from the Latin, auricular- external ear of animals, forma- form, figure or shape, in allusion to the shape of the legume. Standard trade name of the plant in Australia is northern black wattle.

The vernacular names include

English	• Australian Wattle.
Hindi	• Bengali babul.
Bengali	• Akashmoni.
Telegu	• Minnumaan, Kondamanu, Seema babul, Maha babul.

Tamil	• Kaththi Karuvel
Kannada	• Aurculis
Marathi	• Australian babul, Akashia.
In other countries	
Papua New Guinea	• Ngarari, Unar
Indonesia	• Kasia
India	• Australian babul
Philippines	• Japanese Acacia
Australia	• Northern Black Wattle

and other names include Dal Moth, Earleaf Acacia, Auri, Earpod Wattle,Wattle, Black Wattle, Tan Wattle, Northern Black Wattle, Darwin BlackWattle, and Papuan Wattle.

Between latitudes 9-16° S and longitudes 130-145° E, Acacia auriculiformis flourishes.[6] The species' natural range extends from sea level to around 400 m, with the majority of its occurrences occurring below 100 m.[7] Acacia auriculiformis is naturally distributed in Australia, Papua New Guinea, and Indonesia.[8] The species is typically found along rivers and streams, notably directly behind mangroves and along saltwater estuaries. Acacia auriculiformis is one of the rare tree species that is as versatile as it is. It has shown to be very effective at restoring and replanting various places.[9]

According to climate, where the mean maximum temperature of the warmest month is 32-38°C and the mean minimum temperature of the coldest month is 12-20°C, plants may be found in hot, humid, and sub-humid zones.[6, 9] It thrives on a variety of soils.[9] In Asia, Africa, and South America, this species is widely recognised as a cultivated tree.[7]

In semi-arid areas of India like Bihar, Orissa, and West Bengal, it has been adopted.[10] In West Bengal in 1946, it was first introduced to India on a plantation-scale. Then, in 1964, it was made available in Karnataka. Its introduction has been expanded to include everything in India, from high terrain to coastal beaches.[11] For the past 25 years, it has been a significant species in afforestation, especially in the southern states of Andhra Pradesh, Kerala, Tamilnadu, Karnataka, Maharashtra, Goa, and Pondicherry. A. auriculiformis is the most significant and commonly planted species in Karnataka when compared to the native species found there because of its adaptability, usefulness, and economic worth.[12]

A. auriculiformis develops into a tree 25–30 m tall in its native environment, with a straight stem predominating for the majority of the tree height.[5] In young trees, the grey or brown bark is more or less smooth, but as the tree ages, it becomes rough and longitudinally fissured. The leaves are thick, leathery, and curled, measuring 10–16 cm long and 1.5–2.5 cm broad. The rachis has been transformed into phyllodes.

Flowers are 3.75–6.25 cm long, fragrant, and range in color from white to rich golden.[10] The pods are about 6.5 cm long and 1.5 cm broad, flat, woody, glaucous, and transversely veined with undulate borders. They are initially straight or curved but become highly twisted and erratically coil on maturity. The seeds are 4-6 mm length and 3-4 mm wide, widely ovate to elliptical in shape. An extended red, yellow, or orange funicle surrounds each seed.[9]

The A. auriculiformis belongs to.[15-17]

Domain	Eukaryota
Kingdom	Plantae
Sub kingdom	Viridiplantae
Infra kingdom	Streptophyta
Phylum	Spermatophyta
Sub phylum	Angiospermae
Class	Magnoliopsida / Dicotyledonae
Sub class	Rosidae
Superorder	Rosanae
Order	Fabales
Family	Fabaceae/ Leguminosae
Division	Tracheophyte
Subdivision	Spermatophytina
Genus	Acacia mill
Species	A. auriculiformis A.Cunn. ex Benth

The synonyms of A. auriculiformis include A. auriculiformis A.Cunn. ex Benth., orth. Var., Acacia moniliformis Griseb. and Racosperma auriculiformae (A.Cunn. ex Benth.), Pedley.[17,18]

A. auriculiformis has a variable blooming and fruiting season; for example, in Australia, the flowering season lasts from April to July, and the matured seeds are ready for harvest 4-5 months later, from August to October. In Malaysia, blooming occurs between February and May, and mature fruit seeds can be harvested between October and April. The flowering season in Java, Indonesia, lasts from March to June, whereas ripe seeds can be harvested in Thailand from August to February. A. auriculiformis blooms in India from December through January, and it bears fruit from February through March. While fruits and blooming occur more frequently in September and October in some parts of India from the months of March to December. A. auriculiformis's bark, leaves, and fruits (pods with seeds and funicles) are frequently employed for a variety of biological processes.[19,20]

3. USES

Traditional and ethnomedicinal importance

Native Australians in Australia treat a variety of illnesses with A. auriculiformis tree as folk medicine. The Australian aborigines employ an infusion of the bark to treat painful eyes and pains, while a decoction of the root is used to treat rheumatism.[21] The tree's seeds are also used to treat skin conditions like allergies, rashes, and itching.[22] This plant is used as an antimalarial remedy by the Ibibio population in Nigeria's Niger Delta region.[6] The numerous components of the A. auriculiformis plant extract and phytoconstituents have been reported to be effective against a variety of illnesses, including microbiological infections, conjunctivitis, pain, rheumatism, candidiasis, and HIV.[23]

4. CHEMICAL CONSTITUENTS[24– 31]

Sl. No	Chemical Constituents/ Phytochemicals		Present in which part
1.	Carbohydrates	• Glucuronic acid, • Methylglucuronic acid, • Galactose, • L-rhamnose • Arabinose.	whole plant. The plant has a high carbohydrate content.
2.	Tannins	12–16% of tannin.	mostly discovered in bark. Tanning content is greater in younger trees.
3.	Anthocyanidins	• Leucodelphinidins • Leucocyanidins	are present in the bark, which redden exceptionally in light.
4.	Flavonoids	Three isomeric flavan 3,4 diols: • (-) teracacidin (2R, 3R,4R)- 4′, 7,8 trihydroxy- 2,3-cis-flavan-3,4-cis-diol, • (-)-isoteracacidin (-)-2,3-cis- 3,4-trans isomer And a crystalline methyl ether derivative of **4′7,8-trihydroxy-2,3-trans flavan-3,4-cis-diol.** Funicles were discovered to contain **(+)- 4',7,8- trihyroxydihydro flavonol** and **(+)- 4',7,8- trihyroxyflavanone.**[15] From the plant's aerial portion, **auriculoside**, a new flavan glycoside, was identified. Auriculoside was identified as **7, 3', 5' - trihydroxy-4'-methoxyflavan-3'-O-β-D glucopyranoside** during the process of elucidating its structure.	Mainly in heartwood.
5.	Saponins	Saponins are unique in nature due to presence of tridesmoside saponins i.e., **Proacaciaside-I**, **Proacaciaside-II** and **acaciamine**. They were isolated from the fruits and their structure have been identified as **acacic acid lactone-3-O-β D glucopyranosyl (1→6)-β-D glucopyranoside (proacaciaside- I)**, **acacic acid lactone-3-O-α-L- arabinopyranosyl (1→2)-β-o-glucopyranoside (proacaciaside-II)** and **acacic acid lactone- 3-O-α-L-arabinopyranosyl (1→6)-2 acetamido-2- deoxy-β-D-glucopyranoside (acaciamine)** Apart from these, two acylated **biglycoside saponins** called **Acaciasides A** and **Acaciasides B** have also been isolated from fruits and their structures have been characterized as **3-O [β-Dglucopyranosyl (1→6) {α-L arabinopyranosyl (1→2)} -β Dglucopyranosyl]- 21-O-{(6′S)-2′-trans 2′, 6′-dimethyl-6′-O- β-D glucopyranosyl-2′ ,7′ –octadienoyl} acacic acid 28-O Lrhamnopyranosyl (l→6) [β-D xvlopyranosyl (l→2)]-β**	Mainly in funicle

		Dglucopyranoside and **3-O-[β-D glucopyranosyl (l 6j {α Larabinopyranosyl (1→2) }-β-D glucopyranosyl]-21-O- [(6′S)-2′ -trans 2′.6′-dimethvl-6′-O-{β-D-xylopyranosyl (l→2)-β-D-glucopyranosyl-2′, 7′ octadienoyl]- acacic acid 28-O-α-L rhamnopyranosyl (l→6) [β-D xylopyranosyl (l→2)]-β-D glucopyranoside** respectively. The seeds of A. auriculiformis are a rich source of Acaciaside-A and Acaciaside-B. A new **triterpenoid saponin** name **3-O-[α Lrhamnopyranosyl-(1→4)]-α-L- arabinopyranosyl-(1→6)-β- D galactopyranosyl-3β, 16α, 21β, 22α, 28 pentahydroxyolean- 12-ene** has been isolated from the methanol extract of the stems of the plant.	

5. DOCKING

Materials and Methods

The docking of the compounds gives the protein-ligand binding energy (ΔG) was performed using Auto Dock Vina[32] as an extension in UCSF Chimera.

The Androgen receptor protein was retrieved from RCSB Protein Data Bank (PDB) (http://www.rcsb.org/pdb), PDB-ID 2PIT in PDB format. The protein has good ligand structure goodness of fit to experimental data. As per docking protocol[33], removal of all water and solvent molecules, co-crystallized residues (if any) and mirror chain (if any) was ensured using UCSF Chimera software. Next part is the protein structure preparation and it is also done in Chimera, the protein structures were prepared by assigning the hydrogen atoms, charges and energy minimization using Dock Prep tool. The charges were assigned as per the AM1-BCC method which quickly and efficiently generates high-quality atomic charges for protein and the charges were computed using ANTECHAMBER algorithm. The energy minimization was performed using swiss pdb (SPDBV) viewer. The target proteins after minimization of energy were then saved in PDB format for future docking purpose.

A grid box chosen as the binding region in such a way that it would cover the protein's active site for the hydrophobic surface of the concave region of protein to fit in properly the hydrophobic surface of the ligand giving the best binding score. For visualising in different formats, we used the software Discovery studio and UCSF Chimera.

RESULTS AND DISCUSSION

The protein ligand binding interactions (Table 1) between the targeted protein PDB-ID 2PIT and the ligands which are the main phyto-compounds of our Medicinal Plant found out using molecular docking. The calculations reveals the highest free energy change for these interaction as ΔG = - 8.4 Kcal/mol of Proacaciaside II for protein target Androgen receptor 2PIT inside a grid box of 17.06 × 16.16 × 10.01 Å with size 30 × 30 × 30 Å along x-, y- and z- axes (Fig. 1). The 2D diagram of the ligand inside the docked site showed it makes strong hydrogen bond with TRP (A-751), THR (A-755), PRO (A-682) and ASN (A-756) (Fig. 2).

Docking results of phyto-compounds with 2PIT

Name	PubChem ID	Docking Score
		2PIT
1) Acaciamine	15812666	-8.3
2) Arabinose	439195	-4.6
3) Auriculoside	442260	-6.6
4) Galactose	6036	-5.2
5) Glucuronic Acid	94715	-5.5
6) Isoteracacidin	12304590	-6.8
7) Leucocyanidin	71629	-8.0
8) Leukoefdin	3081374	-7.9
9) L-Rhamnose	25310	-5.3
10) Methylglucuronic acid	22868496	-5.9
11) Proacaciaside I	102446075	-8.3
12) Proacaciaside II	102446076	-8.4
13) Teracacidin	44187798	-6.8

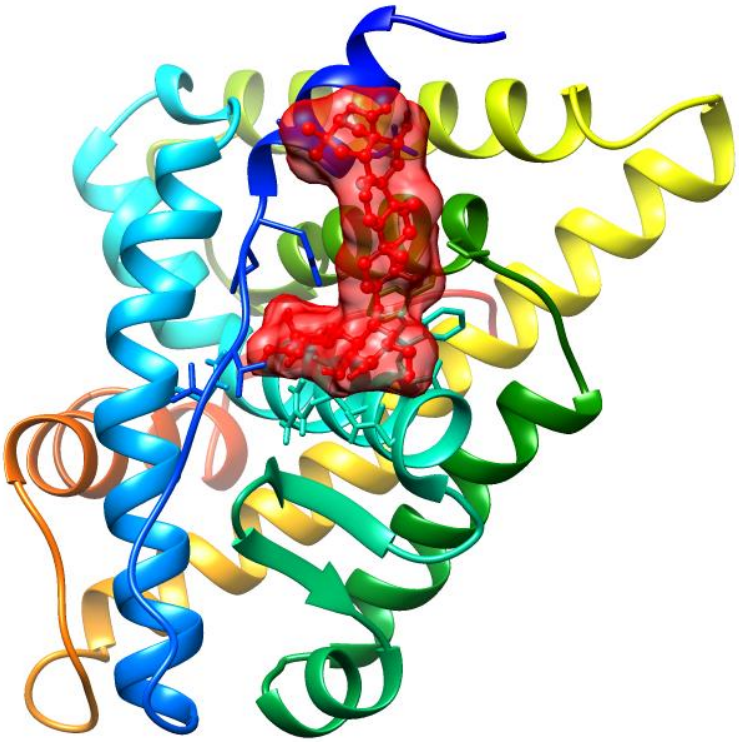


Fig. 1: Docking Pose of Proacaciaside II.

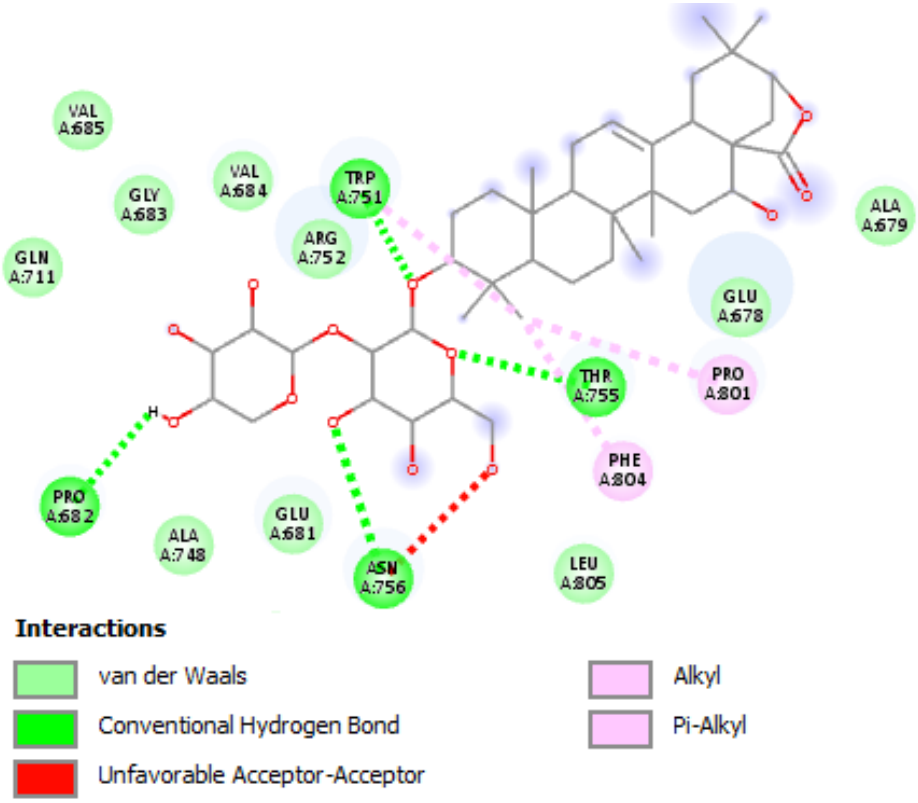
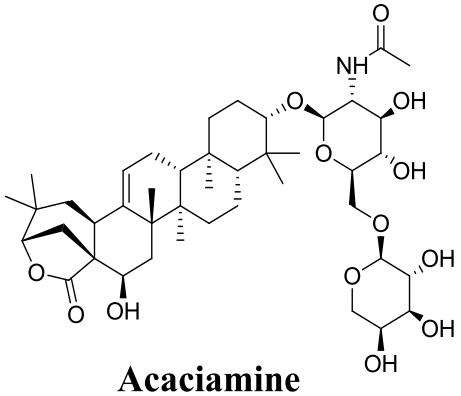
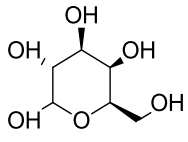


Fig. 2: 2D Diagram of Proacaciaside II in the Binding Site of 2PIT.

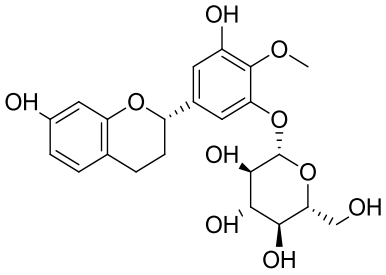
Structures



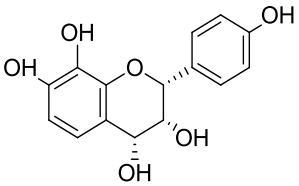
Acaciamine



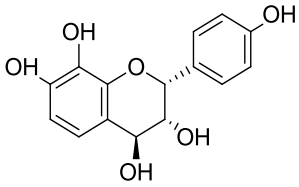
Galactose



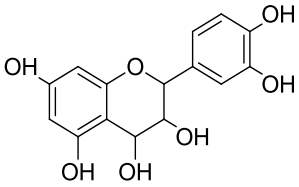
Auriculoside



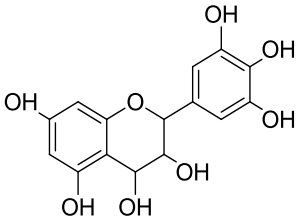
Teracacidin



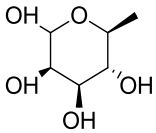
Isoteracacidin



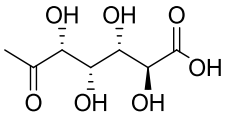
Leucocyanidin



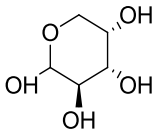
Leukoefdin



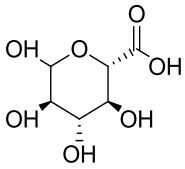
L-Rhamnose



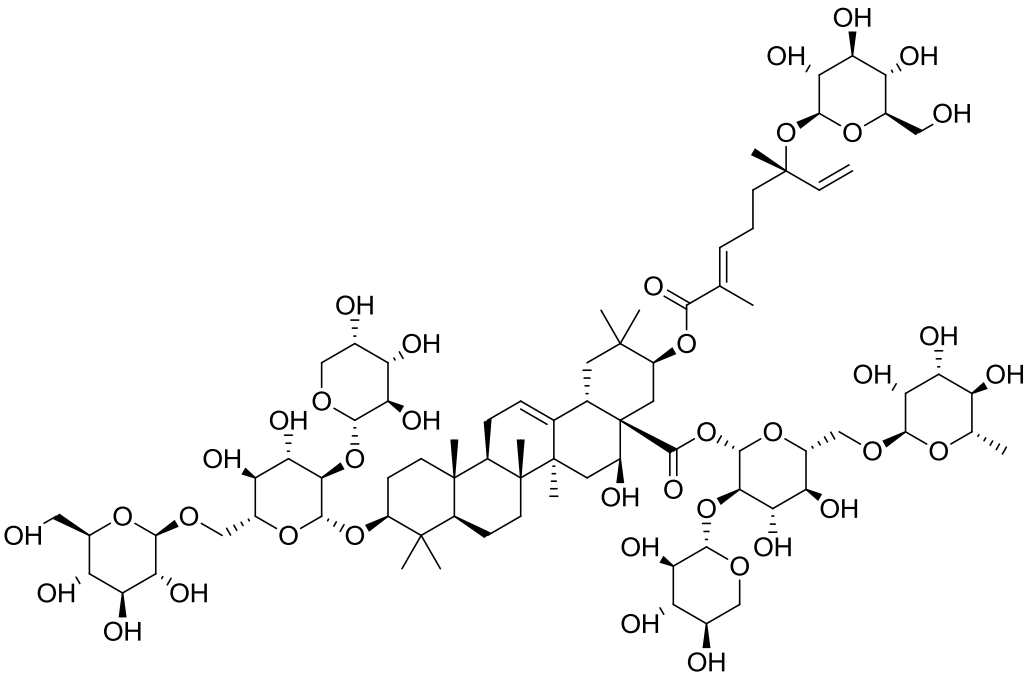
Methylglucuronic acid



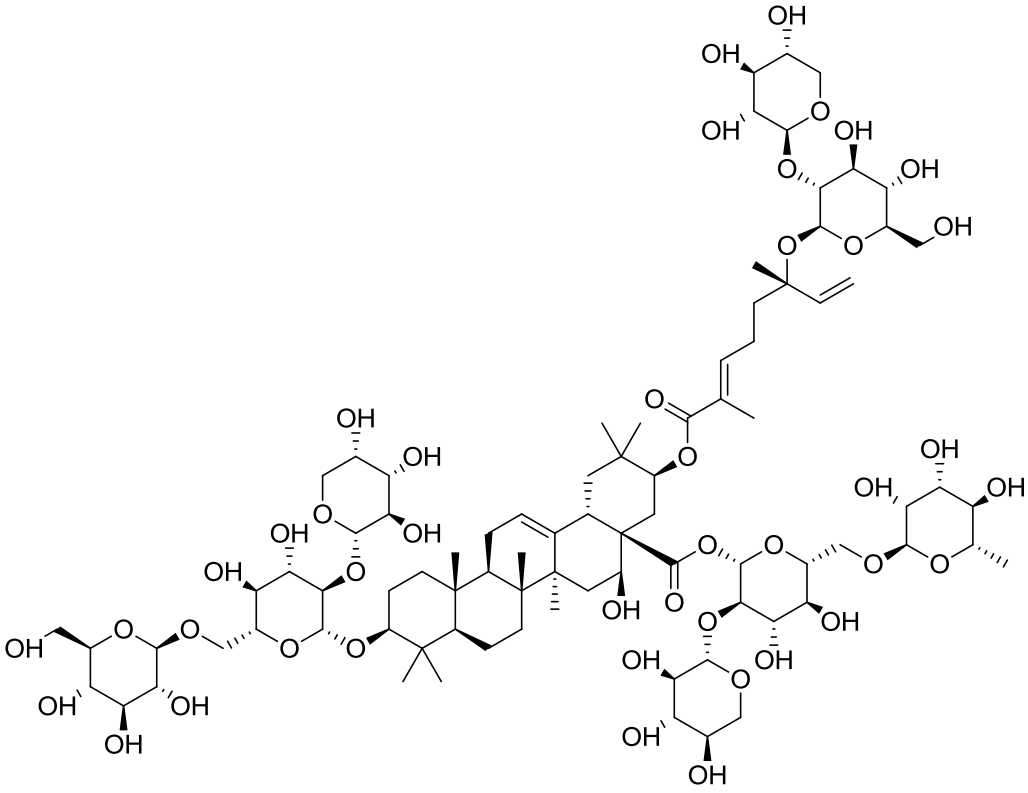
Arabinose



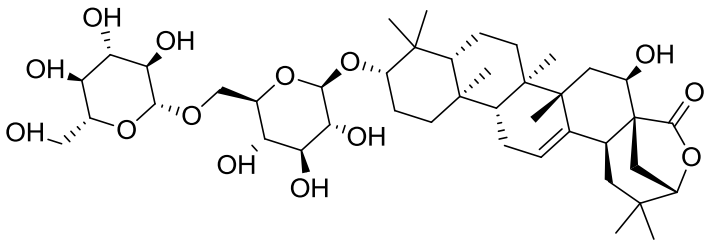
Glucuronic Acid



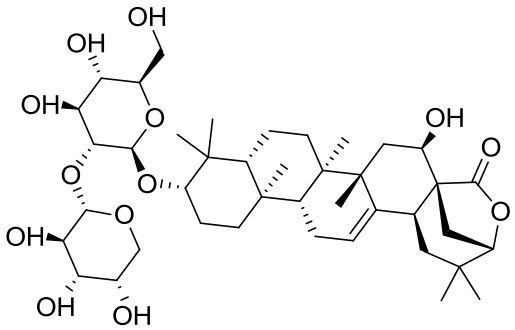
Acaciaside A



Acaciaside B



Proacaciaside I



Proacaciaside II

6. PHARMACOLOGICAL ACTIVITIES:

Pharmacological activities of A. auriculiformis A.Cunn. ex Benth.

Sl. No	Pharmacological Activities	Part used	Extract/ Fraction/ Phytoconstituent tested	Dose tested	Stains/ animal Used	In vivo/ In vitro	References
1	Antioxidant	Heartwood	Methanol, diethyl ether, ethyl acetate, n butanol extracts, isolated compounds (3,4',7,8-tetrahydroxyflavanone, 4',7,8-trihydroxyflavanone, and teracacidin)	0.1, 1.0, and 10.0 mg/mL		In Vitro	[34]
		Bark Powder	Ethyl acetate, methanol, acetone, water extract/fractions, crude extract	10-150 µg/mL, 10-700 µg/mL			[4,35,36]
		Leaves and flowers	Ethanolic extract	1-100 µg/mL			[37]
		Bark and empty pods	Petroleum ether and acetone extracts	1 mL of 1 mg/mL			[38]
		Leaves and bark	Ethyl acetate, methanol, and n-hexane extract	0.1 mL of 1 mg/mL			[39]
		Bark	Ethanolic extract	1.75, 7.80. 7.95 µg/mL			[40]
		Seed	Raw, Dry heated and pressure-cooked extract	900 µg/mL			[41]
		Fruit	Methanolic extracts	1 mg/mL			[42]
		Leaves	Water, Chloroform, Petroleum ether, ethyl acetate and ethanolic extract	25, 50, 75, 100, 125, 150 µg/mL			[43]
2	Antifungal	Funicles	Acaciaside A and Acaciaside B	300 µg/mL	Fungal stains (Aspergillus ochraceous and Curvularia lunata fungal stains)	In vitro	[5,34]
		Heartwood	Methanol, diethyl ether, ethyl acetate, n butanol extracts, isolated compounds (3,4',7,8-	0.1, 1.0, and 10.0 mg/ mL	Fungal stains (Phellinus noxius, Phellinus		

			tetrahydroxyflavanone, 4',7,8-trihydroxyflavanone, and teracacidin)		badius)		
3	Antimicrobial	Funicles	Acaciaside A and B isolated phytoconstituents (Two acylated bisglycoside saponins)	700 µg/mL	Bacterial stains (Bacillus megaterium, Salmonella typhimurium, and Pseudomonas aeruginosa)	In vitro	[5,37,44,45,46,47]
		Flowers and leaves	Ethanolic extract	1 mg/ 100 µL	Bacterial strains (four Gram-negative bacteria i.e., Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, and Enterobacter aerogenes and four Gram positive bacteria i.e., Bacillus cereus, Micrococcus luteus, methicillin-sensitive Staphylococcus aureus, and two strains of methicillin-resistant Staphylococcus aureus and clinical strain)		
		Pods	Aqueous extract synthesized silver nanoparticles	10.8, 14.4, 21.6, 30.8 µg/mL	Gram-positive (Bacillus cereus and Staphylococcus sps.) and Gram-negative organisms (Wild-type Escherichia coli BW 25113 and Klebsiella sps.)		
		Seed pods	Methanolic and ethanolic extract	5, 10, 15, 20 µg/mL	Thirteen strains of Gram positive and Gram negative bacteria (Staphylococcus aureus, Rhodococcus, Bacillus subtilis, Listeria monocytogenes, Escherichia coli, Salmonella typhi, Shigella dynsenteriae, Klebsiella pneumoniae, Salmonella entrica serovar typhimurium, Arizona, Vibrio		

					cholera, Pseudomonas aeruginosa, Acinetobacter boumanii)		
		Phyllodes	Methanolic extract	2 and 6 mg/ mL	Three Gram-positive bacteria (Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus) and three Gram negative bacteria (Klebsiella pneumoniae, Escherichia coli, Pseudomonas aeruginosa)		
		Root and Bark	Hydroalcoholic root and bark extracts	10 mg/mL	Bacteria (Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Proteus mirabilis) and fungi (Aspergillus niger, Candida albicans, Penicillium luteum, Mucar spinescens)		
4	Antimicrobial and antifungal	Bark	Ethanolic extract	100 mg/ mL	Bacterial Stains (Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis) and fungal stains (Aspergillus niger and Candida albicans)	In vitro	[40,43]
		Leaves	Water, chloroform, petroleum ether, ethyl acetate and ethanolic extracts	1,2,3,5 and 6 mg/mL	Eight bacterial (Bacillus subtilis, Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella enteric, Shigella flexneri, Staphylococcus aureus, Vibrio cholera) and three fungal stains (Aspergillus niger, Candida albicans, Cryptococcus sp)		
5	Antimalarial	Leaves	Ethanolic extract	350- 1050 mg/kg/day	Mice	In Vivo	[6]
6	Cestocidal	Funicles	Ethanolic extract and saponins	Ethanolic extract (300 mg/Kg/day)	A Single cysticercoid of Hymenolepis	In Vivo	[8]

				and saponins (200 mg/kg/day)	diminuta; rats		
7	Antifilarial	Funicles	Two triterpenoid saponins, acaciaside A and acaciaside B isolated from the ethanolic funicles extract	4 mg/mL, 100 mg/kg	Microfilaria of Setaria cervi, rats	In vitro, In vivo	[7,48]
			Ethanolic extract	150 mg/kg/day for 45 days	Pariah dog; Dirofilaria immitis		
8	Larvicidal	Leaves	Water, chloroform, petroleum ether, ethyl acetate and ethanolic extracts	2,4,6,8,10 µg/mL	Aedes albopictus and Culex quinquefasciatus	In vitro	[43]
9	Pesticidal	Bark	Acetone and water extract	1,5,25,125, 625 ppm	Bactrocera cucurbitae (Coquillett)	In Vitro	[49]
10	Learning and memory	Leaves	Ethanolic extract	200 mg/kg and 400 mg/kg	Rats (Wistar strain)	In Vivo	[50]
11	CNS depressant	Aerial parts	Butanol extract fraction (Further resolved into ethyl acetate soluble and insoluble fractions)	50% extract	Mice	In vitro	[13,14]
12	Antimutagenic and Chemopreventive	Bark	Chloroform and acetone extract	2.5 X 10^3 – 0.01 X 10^3 µg/0.1 mL	Bacterial stains and Balb/C female mice	In vitro, In vivo	[9]
13	Antidiabetic	Bark and empty pods	Petroleum ether and acetone extract	2.5 and 50 µg	-	In Vitro	[38,11]
				100, 200, 400 mg/kg	Mice	In vivo	
14	Hepatoprotective	Bark and pods	Petroleum ether and acetone extract	100 – 200 mg/Kg for 7 days	Sprague–Dawley albino rats	In vivo	[11]
	Wound healing	Bark	Ethanolic and aqueous extract ointment	5% w/w ethanol extract ointment	Rats	In vivo	[12]
	Spermicidal	Seed	Ethanolic extract, triterpenoid saponins (Acaciaside A and Acaciaside B)	0.35 mg/mL	-	In Vitro	[10,51]
			n-Butanol extract, triterpenoidal saponins-acaciaside B enriched isolated fraction	120 µg/mL		In vitro	

7. TOXICITY STUDIES

Acute toxicity studies

Acute toxicity experiments were conducted using A. auriculiformis ethanolic leaf extracts at doses of 500–5,000 mg/kg. The outcomes demonstrated dose-dependent physical toxicity symptoms, including death, writhing, gasping, palpitations, reduced respiratory rate, and body and limb bone. The extract's LD50 in mice administered intraperitoneally was determined to be 3,741.7 mg/kg. The mice who had received extract doses of 4,000 mg/kg and above were discovered to be dead.[6] According to Test Guideline 425 of the Organization for Economic Cooperation and Development (OECD), the acute toxicity investigation was conducted on adult female rats (Up and Down Procedure). Following the initial 30 minutes of treatment, each animal received a single dosage of the ethanolic extract of leaves at a maximum concentration of 2,000 mg/kg, and their general behaviour as well as mortality were monitored. The first four hours received extra attention for a total of 14 days. Up to a dosage of 2,000 mg/kg, the ethanolic extract of leaves was determined to be safe.[50] According to OECD guideline No. 402, tests on acute dermal toxicity were conducted on the ethanolic and aqueous extracts of A. auriculiformis bark extract to determine their therapeutic dosage potential. On the mice's shaved back, an ointment was administered that contained total aqueous and ethanolic extracts at the greatest concentration of 12% (w/w). No changes in general behaviour or appearance, as well as weight reduction, etc., were seen in the observations. It was determined that the extract-containing ointment was safe up to the chosen maximum dose of 12% (w/w).[12] A. auriculiformis' hydroalcoholic root and bark extracts were examined for their acute toxicity tests, and the estimated LD50 value was determined to be between 500 and 1000 mg/kg with tonic and clonic convulsions as the symptoms.[47] The methanolic leaf extract of A. auriculiformis was used in the toxicity experiments on Gambusia affinis, a mosquito predator and aquatic creature (fish). According to recent research, the methanolic leaves extract of A. auriculiformis can be utilised as a powerful larvicide. This toxicity study was conducted to evaluate the herbal larvicide's biosafety

against the malaria and Japanese encephalitis vectors Aedes albopictus and Culex quinquefasciatus. According to the findings, the LC50 and LC90 values were 1 670 and 3 450 g/mL, respectively. At LC50 and LC90 levels, the swimming and survival activities of the studied organism showed no appreciable changes.[43]

Cytotoxicity studies

In comparison to the other fractions, the leaf and bark methanolic fractions of A. auriculiformis were shown to have the highest cytotoxic activity (Brine Shrimp Lethality Bio-assay), with LC50 values of 0.55 and 0.79 g/mL, respectively. Additionally, the leaf fraction's ethyl acetate LC50 value was 0.95 g/mL. Standard vincristine sulphate was found to have an LC50 value of 0.52 g/mL.[40]

8. CONCLUSION

Acacia auriculiformis is the oldest and the most popular type of plant with multiple numerous therapeutic properties like antioxidant, antimicrobial, antifungal, antimalarial, cestocidal, antifilarial, larvicidal, pesticidal, learning and memory, CNS depressant, antimutagenic and cancer preventive, antidiabetic, hepatoprotective, wound healing and spermicidal effects. In Australian culture, the clears from Acacia auriculiformis are used as a folk medicine to cure rheumatism, itching, allergies, and itchy eyes as well as other skin conditions. It is also used as a traditional medicine in Nigeria to treat HIV and candidasis. It has numerous phytoconstituents. Hence, there is an immunomodulation impact that will definitely appear. But in order to identify the same, investigation work and clinical trials ought to be done to reflect the above-mentioned impacts on human beings.

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