



Asian Pacific Journal of Tropical Biomedicine

Journal homepage: www.apjtb.org



doi: 10.4103/2221-1691.250263

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A comprehensive review on phytopharmacological investigations of *Acacia auriculiformis* A.Cunn. ex Benth.

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ARTICLE INFO

Article history:

Received 14 November 2018

Revision 15 December 2018

Accepted 6 January 2019

Available online 21 January 2019

Keywords:

Acacia auriculiformis

Pharmacognostical

Phytochemical

Pharmacological

ABSTRACT

Acacia auriculiformis A.Cunn. ex Benth. is a perennial shrub having a wide range of medicinal potentials and is widely distributed throughout the world. It is being used traditionally to overcome various medical complications like sore eyes, aches, rheumatism, allergy, itching, and rashes. Besides, *Acacia auriculiformis* has been proven for many pharmacological activities like central nervous system depressant activity, antioxidant, antimicrobial, antimalarial, anti-filarial, cestocidal, antimutagenic, chemopreventive, spermicidal, wound healing, hepatoprotective and antidiabetic activity due to its low toxicity ($LD_{50} = 3741.7$ mg/kg) and high efficacy. In addition, various phytochemical investigations reveal the presence of chief constituents as flavonoids (Auriculoside) and triterpenoid saponin glycosides (acaciasides- acaciaside A & B) in different parts of this plant. Since many years researchers have been carrying out various studies on this medicinal important shrub to elicit the various biological activities. This review attempts to highlight the pharmacognostical, phytochemical and pharmacological observations from 1965 to 2018 retrieved from SciFinder, Scientific journals, books, Google Scholar, and botanical electronic database websites. The various plant extracts evaluated for different pharmacological activities showed significant efficacy. Bioactive phytoconstituents isolated from various parts of the plant are highlighted. Pharmacognostical standardization of the plant done with various standard parameters is also reported. The low toxicity of this plant and the presence of major bioactive phytoconstituents like flavonoids and triterpenoid saponin glycosides are responsible for a therapeutic remedy for various diseases and pharmacological activities respectively. This review provides exhaustive information about the pharmacognostical, phytochemical, and pharmacological investigations of *Acacia auriculiformis* till date.

1. Introduction

The evolution of human history shows the evidence that people are using traditional medicine for therapeutic purpose. The recent reports from the World Health Organization (WHO) claim that 70%-

80% population is primarily dependent on animals and plant-based medicines because of limited or no access to medical services. The drugs obtained from wild plants and animals are not only used as

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Foundation project: This work was financially supported by University Grants Commission-Basic Scientific Research (UGC-BSR) [Award Letter No. F.25-1/2013-14(BSR)/7-36/2007(BSR)].

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How to cite this article: Rangra NK, Samanta S, Pradhan KK. A comprehensive review on phytopharmacological investigations of *Acacia auriculiformis* A.Cunn. ex Benth. Asian Pac J Trop Biomed 2019; 9(1): 1-11.

traditional medicines but also as raw materials in the formulation of modern allopathic and herbal preparations[1].

Acacia auriculiformis (*A. auriculiformis*) A.Cunn. ex Benth. belonging to family Fabaceae, is a straight, medium-sized, deciduous or evergreen tree, potentially accomplishing 30 m tallness, and is normally found in the roadsides and parks of India. The generic name of acacia is derived from the Greek word 'akis' which means a spike or a point. Whereas the Latin word 'auricula' implies outside ear of creatures which is a particular name through the word 'forma' implying frame, figure or shape. The tree is native to Australia and was first introduced to India in 1946 in West Bengal[2]. The tree is rich in glucuronic acid, methylglucuronic acid, arabinose, rhamnose and galactose[3]. It is reported that plant has shown various pharmacological activities like antioxidant[4], antimicrobial[5], antimalarial[6], antifilarial[7], cestocidal[8], antimutagenic, chemopreventive[9], spermicidal[10], hepatoprotective[11], wound healing[12] and antidiabetic activity[11]. The central nervous system (CNS) depressant activity was observed in a flavan glycoside known as auriculoside isolated from *A. auriculiformis*[9,13,14].

The main objective of the review was to highlight the updated pharmacological and phytochemical investigations about *A. auriculiformis* plant till date. The most relevant and exhaustive literature search was made using keywords "A. *auriculiformis* pharmacology", "A. *auriculiformis* phytochemistry", "A. *auriculiformis* patents" in SciFinder, PubMed, Scopus, and Google Scholar databases. The literature search results revealed articles from 1965 to 2018. Thereafter pharmacological and phytochemical related specific articles were carefully screened, selected and reviewed without any chronological restriction for the compilation of the present manuscript.

1.1. Taxonomic and botanical description

The botanical name of *A. auriculiformis* is *A. auriculiformis* A.Cunn. ex Benth. and the vernacular names include akashmoni in Bengali, Australian wattle in English, Bengali babul in Hindi, minnumaan, kondamanu, seema babul, maha babul in Telugu, kaththi karuvel in Tamil, aurculus in Kannada, Australian babool, akashia in Marathi and other names include Dal Moth, Earleaf Acacia, Auri, Earpod Wattle, Wattle, Black Wattle, Tan Wattle, Northern Black Wattle, Darwin Black Wattle, and Papuan Wattle. The *A. auriculiformis* belongs to domain Eukaryota, kingdom Plantae, subkingdom Viridiplantae, infrakingdom Streptophyta, phylum spermatophyte, subphylum angiospermae, class magnoliopsida/dicotyledonae, subclass rosidae, superorder rosanae, order fabales, family fabaceae/leguminosae, division tracheophyte, subdivision spermatophytina, genus acacia mill, and species *A. auriculiformis* A.Cunn. ex Benth[15–17].

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]. The flowering and fruiting season of *A. auriculiformis* varies from region to region as in Australia flowering season occurs from April to July and

ripened seeds are available after 4-5 months later *i.e.* in August-October. In Malaysia flowering is observed from February to May and ripened seeds from fruits can be collected from October to April. In Java, Indonesia flowering season lies from March to June while in Thailand mature seeds can be collected between August and February. In India, the flowering season of *A. auriculiformis* takes place in the months of December to January and fruiting in February to March. Whereas in some regions of India flowering and fruiting take place in the months of March to December but more in September to October. The bark, leaves, and fruits (pods with seeds and funicles) parts of *A. auriculiformis* are widely used for various biological activities[19,20].

1.2. Habitat and ecology

A. auriculiformis usually occupies terrestrial habitat and is native to Australia, Indonesia, and Papua New Guinea. In India, it is distributed throughout the states except for Jammu & Kashmir, Sikkim, and Arunachal Pradesh. This is because of its ability to grow in poor soils, drought resistance, seasonal tolerance of waterlogged soils and fast growth[19,20].

1.3. Traditional and ethnomedicinal importance

In Australia, *A. auriculiformis* tree is used as a folk medicine by the natives for various diseases. For the treatment of sore eyes and aches, a decoction of the root is used for rheumatism treatment and bark infusion is used by the Australian aborigines[21]. The seeds of the tree are also used as skin ailment in various diseases like itching, allergy, and rashes[22]. The Ibibio community of Niger Delta region in Nigeria uses this plant as antimalarial medicine[6]. The various parts of *A. auriculiformis* plant extract and phytoconstituents are found to be useful in various diseases like candidiasis, rheumatism, conjunctivitis, pain, anthelmintic, human immunodeficiency virus (HIV), and microbial diseases[23].

1.4. Pharmacognostical standardization studies

The pharmacognostical standardization is an important aspect to control the authenticity of plants. It helps to control adulteration and ensures the quality of plants to accept them for worldwide use in trade and medicine system. The morphological or macroscopic studies help in identification of visual aspects of plant properties like organoleptic characters (color, odor, taste), shape, size *etc.* Sharma *et al.*, in 2017 reported pharmacognostical standardization studies of *A. auriculiformis*[24]. Some of the important characteristics are highlighted here. The height of the *A. auriculiformis* tree is 35 to 40 feet and it spreads up to 25 to 35 feet. The crown has irregular uniformity, round shape, and moderate density. It has a fast growth rate and medium texture. The leaves of the tree are of the green and simple type with the alternate arrangement. The leaves have an entire margin with linear shape and parallel venation. The leaves

are evergreen with broadleaf evergreen persistence. The length of the leaf blade is 10.5 to 20.6 cm. There is no color change in fall color of leaves without showy fall characteristic. The flowers are yellow colored with showy characteristics. The fruits are 2.5 to 7.6 cm long and brown in color with an irregular shape. The fruit is characterized as dry and hard covering with no showy and wildlife attraction. The fruits and flowers also show litter problem. There is only one trunk with no thorns. The branches are droop, typical with no showy characteristics. The trunk and branches are pruned for strong structure requirement and are also susceptible to breakage. The current year twig is green colored with a thin thickness. The bark of *A. auriculiformis* has a 5-8 mm thickness with flat pieces and fibrous fracture. The color of the young fresh bark is grey with smooth external surface whereas that of mature bark is dark grayish in color with the rough external surface, longitudinal and transverse striations. The bark has a smooth internal having light color with few dark brown patches usually seen in mature barks. The odor of the bark is characterized with non-bitter taste[24].

2. Phytochemical constituents

The phytoconstituents isolated and reported till date in *A. auriculiformis* are discussed in this section.

2.1. Flavonoids

Several flavonoids are reported in the literature from *A. auriculiformis* extracts. In the early 60's, a new flavan-3,4-diol was isolated from the heartwood of *Acacia auriculaeformis* by paper ionophoresis[25]. A new flavan glucoside i.e. an auriculoside (I, Glc = *b-D*-glucopyranosyl) or 7,3',5'-trihydroxy-4'-methoxyflavan 3'-glucoside was isolated from *A. auriculiformis* and it showed 80% CNS depressant activity[13,14]. Isolation of two constituents quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4H-chromen-4-one] and epicatechin [(2R,3R)-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5,7-triol] was reported from the bark of *A. auriculiformis* A.Cunn[26]. Two new glycosides named as proacaciaside I and proacaciaside II having anti-filarial activity were separated from the fruits of *A. auriculiformis*[27].

2.2. Saponins

Various isolated saponins were reported from the *A. auriculiformis*. A new triterpenoid trisaccharide {acacic acid lactone-3-*O*-*b-D*-glucopyranosyl (1→6)-[*a-L*-arabinopyranosyl (1→2)]-*b-D*-glucopyranoside} was isolated from the seeds of *A. auriculiformis*[28]. A mixture of two partially characterized triterpenoid saponins acaciaside A and acaciaside B with the aglycon structure of acacic acid lactone was separated from *A. auriculiformis* and *in vitro* sperm-immobilizing activity confirmed the efficacy of the isolated compounds[10]. A new triterpenoid saponin was isolated

from the aqueous ethyl alcohol extract of the legumes of *A. auriculiformis* and characterized it as 3-*O*-{[*b-D*-xylopyranosyl(1→3)-*b-D*-xylopyranosyl(1→4)-*a-L*-rhamnopyranosyl(1→2)]-[*a-L*-rhamnopyranosyl(1→4)]-*b-D*-glucopyranosyl}-3,16,21-trihydroxyolean-12-en-28-oic acid[29]. Two novel acylated triterpenoid bisglycosides separated from the fruits of *A. auriculiformis* were reported[30]. Two triterpenoid saponins, acaciaside A and acaciaside B were separated from the funicles of *A. auriculiformis* and studied for their antifilarial effect. Isolated saponins (acaciasides- acaciaside A, acaciaside B and an acylated triterpenoid bisglycoside designated acaciaside C) from fruits of *A. auriculiformis* were screened for their anti-filarial activity and results were found to be significant[27]. Isolation of new triterpenoid saponins proacaciaside-I, proacaciaside-II, acaciaside and acaciamine from the fruits of *A. auriculiformis* was reported and characterized as acacic acid lactone 3-*O*-*b-D*-glucopyranosyl (1→6)-*b-D*-glucopyranoside, acacic acid lactone 3-*O*-*a-L*-arabinopyranosyl (1→2)-*b-D*-glucopyranoside and acacic acid lactone 3-*O*-*a-L*-arabinopyranosyl (1→6)-2-acetamido-2-deoxy-*b-D*-glucopyranoside[31]. A new triterpenoid saponin-3-*O*-[*a-L*-rhamnopyranosyl-(1→4)]-*a-L*-arabinopyranosyl-(1→6)-*b-D*-galactopyranosyl-3b,16a,21b,22a,28-pentahydroxy-olean-12-ene along with known compound corosolic acid was separated from the stem of *A. auriculiformis*[32].

2.3. Carbohydrates

A. auriculiformis tree is enriched with certain carbohydrates like glucuronic acid, methylglucuronic acid, galactose, *L*-rhamnose and arabinose. An acidic polysaccharide was isolated from the defatted seeds of *A. auriculiformis* in which hydrolysis gave *L*-arabinose, *D*-xylose, *D*-galactose, *D*-glucose, and *D*-glucuronic acid[33].

2.4. Tannins

The bark of *A. auriculiformis* is found to contain tannins (12%-16%). The content is higher in younger trees[34].

2.5. Anthocyanidins

The bark of *A. auriculiformis* contains leucodelphinidins and leucocyanidins which show red color in presence of light[35].

3. Pharmacological activities

3.1. Antioxidant activity

Comparison of antioxidant activity of *Acacia mangium* (*A. mangium*) and *A. auriculiformis* was done in the heartwood extracts using the strong DPPH radical scavenging activity method. It was found that *A. auriculiformis* possessed equivalent antioxidant activity

when the comparison was done in both plants[36]. Antioxidant potential or free radical-scavenging activity was performed in ethyl acetate, methanol and acetone extract/fractions of bark powder of *A. auriculiformis* A. Cunn. In DPPH, chelating power, lipid peroxidation, site-specific and non-site specific deoxyribose scavenging assays, the percent inhibitions observed were 71.2%, 73.66%, 83.37%, 75.63% and 72.92% respectively at maximum concentration (10-150 µg/mL) tested using water fraction of ethyl acetate bark extract. Whereas in acetone bark extract/fractions of acetone, the maximum inhibitory percentages obtained were 72.3% (DPPH assay), 91.7% (deoxyribose assay), 1.63% (reducing power assay), 83.3% (chelating power assay) and 70.9% (lipid peroxidation assay) at a concentration range of 10-700 µg/mL. However, compared with crude extract, the fraction (ethyl acetate and water fraction) was found to exhibit good scavenging response in DPPH (72.0%), reducing power (1.76%), site-specific (88.0%) and non-site specific (93.6%) hydroxyl radical scavenging assay in increasing (and decreasing) order of solvent polarity at maximum concentration (1-100 µg/mL). On fractionating the extract, the scavenging activity was found to be increased[4,37,38]. A mild anti-oxidant activity was observed in ethanolic extract of flowers and leaves of *A. auriculiformis* using DPPH radical scavenging assay [IC₅₀ of flowers (152 ± 13) µg/mL and leaves (161 ± 30) µg/mL][39]. Role of phenolics as antioxidants obtained from the bark and empty pods of *A. auriculiformis* was studied and observations showed that both bark and empty pods can be used for the preparation of antioxidant/nutraceutical supplement formulations[40]. A comparative antioxidant study was performed on the leaves and the bark extract of *A. auriculiformis* and concluded that ethyl acetate fraction of bark possessed the highest DPPH scavenging assay activity (IC₅₀ value of 7.80 µg/mL) as compared to methanolic leave extract (IC₅₀ value of 7.95 µg/mL). The *n*-hexane root fraction was found to have the highest nitrogen oxide scavenging activity (IC₅₀ value of 1.75 µg/mL) than the ethyl acetate fraction of leaves (IC₅₀ value of 3.35 µg/mL)[41]. Anti-oxidant activity in ethanolic bark extract of *A. auriculiformis* was reported at a concentration of 900 µg/mL *i.e.* more than 50% of inhibition when compared to the standard[42].

A comparison of the antioxidant activities of *A. auriculiformis*, *Acacia ferruginea* and *Cajanus cajan* seed extracts (raw, dry heated and pressure cooked) was done using *in vitro* methods such as DPPH assay, reducing power assay, hydroxyl radical assay, ABTS assay, linoleic acid emulsion system assay, metal chelation and antihemolytic activity assays. All extracts were found to be dose-dependently active in DPPH, reducing power and hydroxyl radical assays. The maximum peroxidation inhibiting activity level was found to be 91.3%-94.1% at the concentration of 1 mg/mL in the final reaction mixture and all the extracts were found to be potent in ABTS and metal chelating assays. The antihemolytic activity was found to be moderate at a concentration of 500 µg of extract tested[43]. The methanolic fruit extract of *A. auriculiformis* was evaluated for its antioxidant potential using DPPH assay and found to be potent (Antioxidant activity=78.9% and total phenolic activity=73.1%) at the IC₅₀ of 0.031 mg/mL[44]. The antioxidant activity using DPPH scavenging assay was performed on water, chloroform, petroleum ether, ethyl acetate and ethanolic leaves extracts of *A. auriculiformis* at the concentrations of 25-150 µg/mL. The observations revealed that ethanol extract was found to be most active against DPPH radical scavenging activity whereas all extracts exhibited activity in the decreasing order as Ethanol>Chloroform>Ethyl acetate >Petroleum ether>Water[45] (Table 1).

3.2. Antifungal activity

Antifungal activity was shown by two isolated acaciaside A and B (acylated bisglycoside saponins) from the funicles of *A. auriculiformis*. The acaciaside A and B inhibited *Aspergillus ochraceous* and *Curvularia lunata* fungal stains at a concentration of 300 µg/mL or less[5]. A comparative study of the antifungal activity of *A. mangium* and *A. auriculiformis* was performed in the heartwood extracts and found that *A. auriculiformis* had greater antifungal activity than *A. mangium*. Both species, rich in compounds 3,4',7,8-tetrahydroxyflavanone and teracacidin, showed significant antifungal activity. *A. auriculiformis* having higher levels of 3,4',7,8-

Table 1

Antioxidant activity of *A. auriculiformis* A.Cunn. ex Benth.

Sr.No	Part used	Extract/fraction/ phytoconstituent tested	Dose tested	<i>In vivo/ Reference in vitro</i>
1.	Heartwood	Methanol, diethyl ether, ethyl acetate, <i>n</i> -butanol extracts, isolated compounds(3,4',7,8-tetrahydroxyflavanone, 4',7,8-trihydroxyflavanone, and teracacidin)	0.1, 1.0, and 10.0 mg/mL	<i>In vitro</i> [36]
2.	Bark powder	Ethyl acetate, methanol, acetone, water extract/fractions, crude extract	10–150 µg/mL, 10–700 µg/mL, 1–100 µg/mL	<i>In vitro</i> [4,37,38]
3.	Leaves and flowers	Ethanolic extract	1 mL of 1 mg/mL	<i>In vitro</i> [39]
4.	Bark and empty pods	Petroleum ether and acetone extracts	0.1 mL of 1 mg/mL	<i>In vitro</i> [40]
5.	Leaves and bark	Ethyl acetate, methanol, and <i>n</i> -hexane extract	1.75, 7.80, 7.95 µg/mL	<i>In vitro</i> [41]
6.	Bark	Ethanolic extract	900 µg/mL	<i>In vitro</i> [42]
7.	Seed	Raw, dry heated and pressure cooked extracts	1 mg/mL	<i>In vitro</i> [43]
8.	Fruit	Methanolic extracts	1 mg/mL	<i>In vitro</i> [44]
9.	Leaves	Water, chloroform, petroleum ether, ethyl acetate and ethanolic extracts	25, 50, 75, 100, 125, 150 µg/mL	<i>In vitro</i> [45]

tetrahydroxyflavanone and teracacidin flavonoids (3.5- and 43-fold higher, respectively) than *A. mangium* suggests that these compounds may find use in heartrot resistance. Moreover, the higher DPPH radical scavenging activity and laccase inhibition imply that the antifungal mechanism of these compounds may involve inhibition of fungal growth by concealing the free radicals produced by the extracellular fungal enzyme laccase[36] (Table 2).

3.3. Antimicrobial activity

The isolated acaciaside A and B (acylated bisglycoside saponins) obtained from the funicles of *A. auriculiformis* possessed moderate antimicrobial activity. The acaciaside inhibited *Bacillus megaterium*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* (*P. aeruginosa*) at 700 µg/mL or higher concentrations of the mixture[5]. The mild antimicrobial activity was observed in ethanolic extracts of flowers and the leaves of *A. auriculiformis* on eight species of bacteria [four Gram-negative bacteria i.e. *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *P. aeruginosa*, and *Enterobacter aerogenes* and four Gram-positive bacteria i.e. *Bacillus cereus*, *Micrococcus luteus*, methicillin-sensitive *Staphylococcus aureus* (*S. aureus*), and two strains of methicillin-resistant *S. aureus* and clinical strain] using disc diffusion (Kirby-Bauer) method. All the extracts showed promising activity against Gram-positive bacteria but were inactive against Gram-negative bacteria because of the presence of outer membrane as a permeability barrier in Gram-negative bacteria[39]. In ethanolic bark extract of *A. auriculiformis*; significant antimicrobial activity was observed. Three bacterial strains *S. aureus*, *P. aeruginosa*, *Bacillus subtilis* (*B. subtilis*) and two fungal stains *Aspergillus niger* (*A. niger*) and *Candida albicans* (*C. albicans*) were used in the study. The highest antibacterial activity shown by the ethanolic extract was against the *P. aeruginosa* (19.54±0.40) mm while the highest antifungal activity was shown towards the *A. niger* (20.62±0.17) mm at higher concentration of 100 mg/mL[42]. The antibacterial property of *A. auriculiformis* pod aqueous extract synthesized silver nanoparticles was tested against Gram-positive (*Bacillus cereus* and *Staphylococcus* sps.) and Gram-negative organisms (Wild-type *E. coli* BW 25113 and *Klebsiella* sps.) using minimum inhibitory concentration method and showed significant results[46]. The antimicrobial activities were observed in the methanolic and ethanolic seed pod extracts of *A. auriculiformis*. Thirteen strains of Gram positive and Gram negative bacteria (*S. aureus*, *Rhodococcus*, *B. subtilis*, *Listeria monocytogenes*, *E. coli*, *Salmonella typhi*, *Shigella dysenteriae*, *K. pneumoniae*, *Salmonella enterica* serovar typhimurium, Arizona, *Vibrio cholera*, *P. aeruginosa*, *Acinetobacter boumanii*) were used and extracts were added with the increasing concentration (5, 10, 15, 20 µg/mL) to the standard nutrient agar media. The seed pod extracts inhibited most of the Gram positive and Gram negative bacteria except *Listeria monocytogenes*, *K. pneumoniae*, *P. aeruginosa* and *Acinetobacter boumanii* which were found to be resistant at 20 µg/

mL of the extracts and simultaneously these four bacteria were found to be resistant to many known antibiotics. The ethanolic extract was observed to be more potent than methanolic extracts against both Gram-positive and Gram-negative bacteria[47]. The antibiotic effect of the methanolic phyllodes extract of *A. auriculiformis* was studied in three Gram-positive bacteria (*S. aureus*, *Streptococcus pyogenes*, *Bacillus cereus*) and three Gram-negative bacteria (*K. pneumoniae*, *E. coli*, *P. aeruginosa*) using agar diffusion antimicrobial bioassay at the concentration of 2 and 6 mg/mL. The results predicted that methanol extract was actively inhibited Gram-positive bacteria (*S. aureus* and *Streptococcus pyogenes*) as well as Gram-negative bacteria (*E. coli*)[48]. The water, chloroform, petroleum ether, ethyl acetate and ethanolic leaves extracts of *A. auriculiformis* were evaluated for their antimicrobial and antifungal activities using agar disc diffusion assay on eight bacterial (*B. subtilis*, *E. coli*, *Proteus mirabilis*, *P. aeruginosa*, *Salmonella enteric*, *Shigella flexneri*, *S. aureus*, *Vibrio cholera*) and three fungal stains (*A. niger*, *C. albicans*, *Cryptococcus* sp). The mean minimum inhibitory concentration values of various tested extracts like ethanolic, ethyl acetate, chloroform, petroleum ether and water extract for bacterial stains were found to be 2 mg/mL, 3 mg/mL, 6 mg/mL, 5 mg/mL, 1 mg/mL whereas for fungal stains the values were 2 mg/mL, 2 mg/mL, 6 mg/mL, 5 mg/mL, 1 mg/mL respectively. The inference showed that the ethanolic, ethyl acetate, and water extracts were found to be active against certain bacteria and fungi[45]. The hydroalcoholic root and bark extracts of *A. auriculiformis* were explored for their antimicrobial activity against various strains of bacteria (*B. subtilis*, *S. aureus*, *E. coli*, *Proteus mirabilis*) and fungi (*A. niger*, *C. albicans*, *Penicillium luteum*, *Mucor spinescens*) using agar streak-dilution method at the concentration of 10 mg/mL. The results showed that the *B. subtilis* and *Proteus mirabilis* growth were inhibited at a concentration of 5-10 mg/mL while *S. aureus* showed the highest antimicrobial activity i.e. growth inhibition at the concentration of 1-1.40 mg/mL. The *E. coli* and fungal stains did not show any inhibition[49] (Table 2).

3.4. Antimalarial activity

A significant antiplasmodial potential was observed in ethanolic leaf extract in *A. auriculiformis* at the dose of 350-1 050 mg/kg/day compared to standard drug chloroquine(5 mg/kg/day). The *in vivo* antimalarial activity was performed in *Plasmodium berghei* infected mice using suppressive schizonticidal activity method. The results showed significant activity ($P<0.05$) which supports its claim of traditional use[6] (Table 3).

3.5. Cestocidal activity

The cestocidal activity was observed in the ethanolic extract of funicles of *A. auriculiformis*. A single cysticeroid of *Hymenolepis diminuta* was inoculated orally to two groups each of 10 rats.

Table 2Antifungal, and antimicrobial activity of *A. auriculiformis* A.Cunn. ex Benth.

Sr.No	Pharmacological activity	Part used	Extract/fraction/ phytoconstituent tested	Dose tested	Stains used	<i>In vitro</i>	Reference
1.	Antifungal	Funicles	Acaciaside A and B	300 µg/mL	Fungal stains (<i>Aspergillus ochraceus</i> and <i>Curvularia lunata</i> fungal stains)	<i>In vitro</i>	[5]
2.	Antifungal	Heartwood	Methanol, diethyl ether, ethyl acetate, <i>n</i> -butanol extracts, isolated compounds (3,4',7,8-tetrahydroxyflavanone, 4',7,8-trihydroxyflavanone, and teracacidin)	0.1, 1.0, and 10.0 mg/mL	Fungal stains (<i>Phellinus noxius</i> , <i>Phellinus badius</i>)	<i>In vitro</i>	[36]
3.	Antimicrobial	Funicles	Acaciaside A and B isolated phytoconstituents (two acylated bisglycoside saponins)	700 µg/mL	Bacterial stains (<i>Bacillus megaterium</i> , <i>Salmonella typhimurium</i> , and <i>Pseudomonas aeruginosa</i>)	<i>In vitro</i>	[5]
4.	Antimicrobial	Flowers and leaves	Ethanol extract	1 mg/100 µL	Bacterial strains (four Gram-negative bacteria i.e. <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , and <i>Enterobacter aerogenes</i> and four Gram positive bacteria i.e. <i>Bacillus cereus</i> , <i>Micrococcus luteus</i> , methicillin-sensitive <i>Staphylococcus aureus</i> , and two strains of methicillin-resistant <i>Staphylococcus aureus</i> and clinical strain)	<i>In vitro</i>	[39]
5.	Antimicrobial and antifungal	Bark	Ethanol extract	100 mg/mL	Bacterial Stains (<i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus subtilis</i>) and fungal stains (<i>Aspergillus niger</i> and <i>Candida albicans</i>)	<i>In vitro</i>	[42]
6.	Antimicrobial	Pods	Aqueous extract synthesized silver nanoparticles	10.8, 14.4, 21.6, 30.8 µg/mL	Gram-positive (<i>Bacillus cereus</i> and <i>Staphylococcus</i> spp.) and Gram-negative organisms (Wild-type <i>Escherichia coli</i> BW 25113 and <i>Klebsiella</i> spp.)	<i>In vitro</i>	[46]
7.	Antimicrobial	Seed pods	Methanolic and ethanolic extract	5, 10, 15, 20 µg/mL	Thirteen strains of Gram positive and Gram negative bacteria (<i>Staphylococcus aureus</i> , <i>Rhodococcus</i> , <i>Bacillus subtilis</i> , <i>Listeria monocytogenes</i> , <i>Escherichia coli</i> , <i>Salmonella typhi</i> , <i>Shigella dysenteriae</i> , <i>Klebsiella pneumoniae</i> , <i>Salmonella enterica</i> serovar typhimurium, Arizona, <i>Vibrio cholera</i> , <i>Pseudomonas aeruginosa</i> , <i>Acinetobacter boumanii</i>)	<i>In vitro</i>	[47]
8.	Antimicrobial	Phyllodes	Methanolic extract	2 and 6 mg/mL	Three Gram-positive bacteria (<i>Staphylococcus aureus</i> , <i>Streptococcus pyogenes</i> , <i>Bacillus cereus</i>) and three Gram-negative bacteria (<i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i>)	<i>In vitro</i>	[48]
9.	Antimicrobial and antifungal	Leaves	Water, chloroform, petroleum ether, ethyl acetate and ethanolic extracts	1, 2, 3, 5, and 6 mg/mL	Eight bacterial (<i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Proteus mirabilis</i> , <i>Pseudomonas aeruginosa</i> , <i>Salmonella enteric</i> , <i>Shigella flexneri</i> , <i>Staphylococcus aureus</i> , <i>Vibrio cholera</i>) and three fungal stains (<i>Aspergillus niger</i> , <i>Candida albicans</i> , <i>Cryptococcus</i> sp)	<i>In vitro</i>	[45]
10.	Antimicrobial	Root and Bark	Hydroalcoholic root and bark extracts	10 mg/mL	Bacteria (<i>Bacillus subtilis</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> , <i>Proteus mirabilis</i>) and fungi (<i>Aspergillus niger</i> , <i>Candida albicans</i> , <i>Penicillium luteum</i> , <i>Mucor spinescens</i>)	<i>In vitro</i>	[49]

On the 20th day, the ethanolic extract (300 mg/kg/day) and the saponins (200 mg/kg/day) were administered orally to each of 10 rats of two groups respectively. After treating with ethanolic extract and saponins; the adult worms were expelled within 5 and 3 days respectively [8] (Table 3).

3.6. Antifilarial activity

The two triterpenoidal saponins, acaciaside A and B isolated from the funicles of *A. auriculiformis* killed 97% microfilaria of *Setaria cervi* in 100 min at 4 mg/mL concentration and 100% of adults in 35 min using *in vitro* procedure. The oral administration of the drug at 100 mg/kg for ten days on *Setaria cervi* adult intra-peritoneally

implanted rats elevated the blood microfilaria count by 1.5-fold after the first phase of treatment. By the end of the third phase of treatment, the microfilaria density was reduced to more than 80%. Saponins showed no toxic effect on rats. This increased microfilaria count suggested that drug formed a very high physiological stress on the adult worms which elevated the rate of discharge of the microfilaria before leading to death. No adult worms were found on the pathological examination of treated rats[7]. The 98%-99% reduction was found in microfilarial density when pariah dogs naturally infected with *Dirofilaria immitis* were treated with an ethanolic extract obtained from funicles of *A. auriculiformis* at the dose at 150 mg/kg/day for 45 days[50] (Table 3).

3.7. Larvicidal activity

The larvicidal activity was performed as per WHO recommended method on malarial and Japanese encephalitis vectors *Aedes albopictus* and *Culex quinquefasciatus* using water, chloroform, petroleum ether, ethyl acetate and ethanolic leaves extracts of *A. auriculiformis*. The results showed that ethanolic extract inhibited the two tested larval species in a dose-dependent manner. The LC₅₀ values obtained for *Aedes albopictus* and *Culex quinquefasciatus* were 6.1 µg/mL and 4.2 µg/mL and whereas the LC₉₀ values observed were 8.5 µg/mL and 9.42 µg/mL respectively[45] (Table 3).

3.8. Pesticidal activity

The inhibitory pesticidal effect of acetone and water bark extracts of *A. auriculiformis* was observed in *Bactrocera cucurbitae* (Coquillett). The different concentrations *i.e.* 1, 5, 25, 125, 625 ppm of bark extract were applied to eggs, larvae, and adults. The results showed elongated total developmental and larval period. In a dose-dependent

manner, the percentage pupation, percent emergence, oviposition, and egg hatching were found to be decreased. So, both the extracts were found to be significant biopesticides and acetone extract was found to be more toxic than water extract due to low LC₅₀ values[51] (Table 3).

3.9. Learning and memory effect

The learning and memory effect was evaluated in ethanolic extract of *A. auriculiformis* leaves in rats. The methods used were passive avoidance and rewarded alteration tests on inbred albino rats of Wistar strain. The results showed dose-dependent improvement in memory and also inhibition of acetylcholinesterase (AChE) enzyme by the ethanolic extract as compared to standard drug rivastigmine. This concluded that plant can be used in dementia[52] (Table 4).

3.10. CNS depressant activity

CNS depressant activity was observed in the butanol extract fraction of aerial parts of *A. auriculiformis* through barbiturate potentiation test in mice. The butanol fraction was further resolved into ethyl acetate soluble and insoluble fractions. The ethyl acetate soluble portion was found to contain a phytoconstituent *i.e.* auriculoside which was responsible for 80% CNS depressant activity[13,14] (Table 4).

3.11. Antimutagenic and chemopreventive activity

Antimutagenic and chemopreventive activities were observed in the chloroform and acetone bark extract of *A. auriculiformis* A.Cunn. and *Acacia nilotica* (L.) Wild. Ex Del. using the Ames antimutagenicity assay in two different strains using both direct-acting (4-nitro-o-phenylenediamine or sodium azide) and indirect-acting (2-aminofluorene) mutagens and the mouse mammary

Table 3

Cestocidal, antifilarial, larvicidal, pesticidal and antimalarial activities of *A. auriculiformis* A.Cunn. ex Benth.

Sr.No	Pharmacological activity	Part used	Extract/fraction/ phytoconstituent tested	Dose tested	Stains/animals used	<i>In vivo/in vitro</i>	Reference
1	Antimalarial activity	Leaves	Ethanolic extract	350-1 050 mg/kg/day	Mice	<i>In vivo</i>	[6]
2.	Cestocidal activity	Funicles	Ethanolic extract and saponins	Ethanolic extract (300 mg/kg/day), Saponins (200 mg/kg/day)	A single cysticeroid of <i>Hymenolepis diminuta</i> ; rats	<i>In vivo</i>	[8]
3.	Antifilarial activity	Funicles	Two triterpenoid saponins, acaciaside A and acaciaside B isolated from the ethanolic funicles extract	4 mg/mL, 100 mg/kg	Microfilaria of <i>Setaria cervi</i> , rats	<i>In vitro, in vivo</i>	[7]
4.	Antifilarial activity	Funicles	Ethanolic extract	150 mg/kg/day for 45 days	Pariah dog; <i>Dirofilaria immitis</i>	<i>In vitro, in vivo</i>	[50]
5.	Larvicidal activity	Leaves	Water, chloroform, petroleum ether, ethyl acetate and ethanolic extracts	2, 4, 6, 8, 10 µg/mL	<i>Aedes albopictus</i> and <i>Culex quinquefasciatus</i>	<i>In vitro</i>	[45]
6.	Pesticidal activity	Bark	Acetone and water extracts	1, 5, 25, 125, 625 ppm	<i>Bactrocera cucurbitae</i> (Coquillett)	<i>In vitro</i>	[51]

Table 4Learning and memory effect, and CNS depressant activities of *A. auriculiformis* A.Cunn. ex Benth.

Sr.No	Pharmacological activity	Part used	Extract/fraction/ phytoconstituent tested	Dose tested	Animals/stains used	<i>In vivo/in vitro</i>	Reference
1.	Learning and memory effect	Leaves	Ethanol extract	200 mg/kg and 400 mg/kg	Rats (Wistar strain)	<i>In vivo</i>	[52]
2.	CNS depressant activity	Aerial parts	Butanol extract fraction (Further resolved into ethyl acetate soluble and insoluble fractions)	50% extract	Mice	<i>In vitro</i>	[13,14]

gland organ culture model [activity evaluation was based on the development of preneoplastic lesions in response to the chemical carcinogen 7,12-dimethylbenz(a)anthracene]. The acetone extract showed potent results suggesting that these plants may contain active chemopreventive agents[9] (Table 5).

3.12. Antidiabetic activity

Previous studies indicated that phenolic compounds were identified from extracts of bark and empty pods of *A. auriculiformis* for their antioxidant, bimolecular protectors and as an anti-diabetic spectrum. The observations revealed that both bark and empty pods can be utilized for the preparation of antioxidant/nutraceutical supplements and in antidiabetic formulations[40]. The protective effect of bark and empty pod extracts of *A. auriculiformis* was observed against alloxan-induced type II diabetes and it was found that the bark and empty pod extracts of *A. auriculiformis* were good therapeutic candidates for diabetes[11] (Table 5).

3.13. Hepatoprotective activity

The bark and pod extracts of *A. auriculiformis* were evaluated for their hepatoprotective effect *i.e.* against paracetamol intoxicated liver injury. The serum obtained from the experimental animals was evaluated for liver function biochemical markers such as alanine transaminase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, and total protein. The evaluation parameters of the tested extract were compared to the standard drug silymarin. The results indicated that bark and pod extracts of *A. auriculiformis* were good therapeutic candidates for liver injury[11] (Table 5).

3.14. Wound healing activity

The *in-vivo* wound healing activity was observed in the ointment which contained ethanolic and aqueous bark extract of *A. auriculiformis* using excision and incision wound models in Swiss albino rats. The study was carried out using histopathological and hydroxyproline content evaluation. The ethanolic bark extract ointment showed higher activity than aqueous bark extract as the former showed a decreased period of epithelialization, increased rate of wound contraction, hydroxyproline content, tensile strength, granulation tissue and fiber formation in comparison

with aqueous bark extract ointment. The activity was due to the presence of phytoconstituents like flavonoids, tannins and phenolic constituents[12] (Table 5).

3.15. Spermicidal activity

The *in vitro* sperm-immobilizing activity was observed in the isolated triterpenoidal saponins acaciaside A and B with the aglycon structure of acacic acid lactone from *A. auriculiformis*. The activity was found to be more potent when compared to the standard Triton X-100 at a dose of 0.35 mg/mL[10]. The *in vitro* sperm immobilizing activity with no mutagenicity was observed in a triterpenoid saponin-acaciaside B enriched fraction isolated from the seeds of *A. auriculiformis* at a minimum effective concentration (MEC) of 120 µg/mL and after the sperm viability tests; the EC₅₀ was found to be 35.20 µg/mL. *Lactobacillus acidophilus* is the major component of vaginal microflora which is responsible for maintenance of vaginal hygiene. It was *in vitro* cultured and showed no malignant effects when fraction was prone to the bacteria at 10×MEC. No mutagenic potential of the fraction was found when tested with Ames tests on different strains of *Salmonella typhimurium* including TA 97a, 98, 100 and 102 which are responsible for mutagen (bp substitution or frameshift) at G-C or A-T bp detection[53] (Table 5).

4. Toxicity studies

4.1. Acute toxicity studies

The ethanolic leave extracts of *A. auriculiformis* at a dose of 500-5000 mg/kg were tested for acute toxicity studies. The results showed dose-dependent physical signs of toxicity such as writhing, gasping, palpitation, decreased respiratory rate, body and limb bone, and death. The LD₅₀ value of the extract through the intraperitoneal route in mice was found to be 3741.7 mg/kg. The mice dosed with 4000 mg/kg and above of the extract were found to be dead[6]. The acute toxicity study was performed on female adult rats according to the Organization for Economic Co-operation and Development (OECD) test guideline 425 (Up and Down Procedure). Animals were treated with the limited dose of 2000 mg/kg of the ethanolic extract of leaves and were observed for mortality and general behavior at least once after the first 30 min during the treatment individually. For a period of 14 days, special attention was given during

Table 5Other activities of *A. auriculiformis* A.Cunn. ex Benth.

Sr.No	Pharmacological activity	Part used	Extract/fraction/ phytoconstituent tested	Dose tested	Animals/stains used	<i>In vivo/in vitro</i>	Reference
1.	Antimutagenic and chemopreventive activity	Bark	Chloroform and acetone extract	2.50×10 ³ –0.01×10 ³ µg/0.1 mL	Bacterial stains and Balb/C female mice	<i>In vitro</i> and <i>in vivo</i>	[9]
2.	Antidiabetic activity	Bark and empty pods	Petroleum ether and acetone extracts	2.5 and 50 µg	–	<i>In vitro</i>	[40]
3.	Antidiabetic activity	Bark and empty pods	Petroleum ether and acetone extracts	100, 200, 400 mg/kg	Mice	<i>In vivo</i>	[11]
4.	Hepatoprotective activity	Bark and pods	Petroleum ether and acetone extract	100–200 mg/kg for 7 days	Sprague–Dawley albino rats	<i>In vivo</i>	[11]
5.	Wound healing activity	Bark	Ethanollic and aqueous extract ointment	5% w/w ethanol extract ointment	Rats	<i>In vivo</i>	[12]
6.	Spermicidal activity	Seeds	Ethanollic extract, triterpenoid saponins (acaciaside A and acaciaside B)	0.35 mg/mL	–	<i>In vitro</i>	[10]
7.	Spermicidal activity	Seeds	<i>n</i> -Butanol extract, triterpenoidal saponins–acaciaside B enriched isolated fraction	120 µg/mL	–	<i>In vitro</i>	[53]

the first 4 hours. The ethanolic extract of leaves was found to be safe up to the dose of 2000 mg/kg[52]. The acute dermal toxicity studies were carried out (according to OECD guideline No.402) on the ethanolic and aqueous extract of bark extract of *A. auriculiformis* to obtain their therapeutic dose potential. The ointment containing ethanolic and total aqueous extracts at the highest concentrations of 12% (w/w) was applied on the shaved back portion of the mice. The observations depicted no change in general behavior or appearance and loss in body weight *etc.* The extract containing ointment was found be safe up to the maximum selected dose of 12% (w/w)[12]. The hydroalcoholic root and bark extracts of *A. auriculiformis* were evaluated for their acute toxicity studies and the estimated LD₅₀ value was found to be in the range of 500-1000 mg/kg and the symptoms observed were tonic and clonic convulsions[49]. The toxicity studies were performed on *Gambusia affinis* *i.e.* a mosquito predator and aquatic organism (fish) using methanolic leaves extract of *A. auriculiformis*. The methanolic leaves extract of *A. auriculiformis* can be used as potent larvicide according to the present studies and this toxicity study was done to observe the biosafety of this herbal larvicide against the malarial and Japanese encephalitis vectors *Aedes albopictus* and *Culex quinquefasciatus*. The observations showed the LC₅₀ and LC₉₀ values obtained were 1670 µg/mL and 3450 µg/mL respectively. The survival and swimming activities depicted no significant changes in tested organism at LC₅₀ and LC₉₀ doses[45].

4.2. Cytotoxicity studies

The leaf and bark methanolic fractions of *A. auriculiformis* were compared with the other fractions and it was found that leaf and bark methanolic fraction had the highest cytotoxic activity (Brine Shrimp Lethality Bio-assay) with LC₅₀ value of 0.55 and 0.79 µg/mL respectively. Furthermore, the LC₅₀ value of ethyl acetate fraction of leaf was 0.95 µg/mL. The LC₅₀ value of standard vincristine sulfate was found to be 0.52 µg/mL[42].

5. Patents

A US patent claimed the potential of acaciaside-B(Ac-B) isolated from *A. auriculiformis* for the prevention of HIV infection and as a vaginal contraceptive[54]. Another US patent titled “Isolation of Dual COX-2 and 5-lipoxygenase inhibitor from *Acacia*” disclosed utilization of flavons isolated from several species in the genus *Acacia* in the formulation of COX-2 and 5-lipoxygenase inhibitors[55]. A US patent filed on 24 February 2004 claimed the novel method invention for diseases and conditions raised from excessive intake of carbohydrates (fructose and glucose drove lipogenesis). A mixture having free B-ring flavonoids and flavans separated from *Scutellaria* and *Acacia* genus was used in the formulation[56]. On 17 April, 2006, a US patent “Formulation of a mixture of free B-ring flavonoids and flavans as a therapeutic agent” was filed by Jia *et al.* who claimed that a formulation containing mixture of free B-ring flavonoids and flavans isolated from *Acacia* genus can be used in the treatment and prevention of platelet aggregation and platelet-induced thrombosis born diseases[57].

6. Conclusion and future prospective

A. auriculiformis is a multipurpose shrub which has been widely used as traditional medicine in several Asian countries. This plant has been used to treat several medical ailments due to its low toxicity and the presence of bioactive phytoconstituents. The present work reveals that *A. auriculiformis* is a treasured source of medicinally important molecules and provides an authentic base for its future use in contemporary medicine. However, due to the restriction of the research areas, there is a need to isolate new phytochemicals and investigate their mechanism of action showing pharmacological effects required to understand traditional use concerns. There are also limitations in clinical studies on isolated bioactive molecules from this plant, which are required to explain pharmacological effects. A

clinical study helps in discovery of potent (specific and effective) medicine and to elaborate the health benefits and side effects.

Conflict of interest statement

All the authors declare no conflict of interest.

Acknowledgements

The authors would like to acknowledge the National Science Library, New Delhi, National Library of India, Kolkata and Central Library, Birla Institute of Technology for providing access to important literature in the preparation of this review article. The authors would also like to acknowledge University Grants Commission-Basic Scientific Research (UGC-BSR) [Award Letter No. F.25-1/2013-14(BSR)/7-36/2007(BSR)] for financial support.

Funding

This work was financially supported by University Grants Commission-Basic Scientific Research (UGC-BSR) [Award Letter No. F.25-1/2013-14(BSR)/7-36/2007(BSR)].

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