

***In vitro* Phytochemical, Larvicidal and Antimicrobial Activities of Gum Arabic Extract**

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Abstract

The Gum Arabic of *Acacia senegal* (GA) has been reported to treat several diseases, such as kidney failure and cardiovascular and gastrointestinal disease. However, scarce investigation has been made into the phytoconstituents of GA. Obtained GA was macerated in water, then GA aqueous extract was subjected to phytochemical analysis using standard protocols and bioactivity screening by different procedures. Antimicrobial screening was performed using the cup-plate diffusion method against four bacterial strains and one fungi strain. The larvicidal activity was evaluated against the third instar of *Culex quinquefasciatus*. The phytochemical analysis showed that GA extract contains high amounts of saponins and alkaloids, moderate amounts of cardiac glycosides, and trace amounts of tannins. GA extract exhibited antimicrobial activity against the test organisms, with different zones of inhibition ranging 0 - 18 mm. The larvicidal activity showed significant perfection with increasing extract dose and exposure period with mortality up to 86.7 %. Results reveal that the crude extract of GA contains important biomolecules which has been proved to have substantial larvicidal and antimicrobial activities.

Keywords: *Acacia senegal*, Antimicrobial, *Culex quinquefasciatus*, gum Arabic, secondary metabolites

Introduction

Gum Arabic (GA) is a natural, water-soluble, multiuse, and edible substance. It is an exudate from injured or intentionally tapped branches of *Acacia senegal* var. *senegal* (L.) Willdenow, or closely related species of *Acacia* (Leguminosae) such as *A. polyacantha* and *A. seyal*; it comes mainly from Sudan. When the duct of the inner bark of *A. senegal* is wounded during dry season, a sticky gummy substance appears and dries to form hard nodules in 3 - 8 weeks [1]. The hand-picked nodules represent a valuable source for rural people in Sudan to cover carbohydrate consumption and to treat many diseases.

In folk medicine, GA is employed to prepare various remedies to cure cough, diarrhea, sore throat, dysentery, gonorrhea, typhoid, urinary tract infections, and intestine inflammation [2-5]. Also, it is used externally to cover inflamed surfaces such as burns, wounds, sore nipples, and nodular leprosy [3,5,6]. Clinically, GA has been used in intravenous injections for the treatment of wound shock during the First World War, to induce antibodies, and to identify blood group antigens [7]. Recent research has asserted that GA possesses antioxidant, nephroprotectant, anti-cancer, anti-malarial, anticoagulant, immunomodulatory, cytoprotective, remineralization, biofungicidal, analgesic, astringent, emollient, liver tonic, antipyretic, anti-asthmatic, and metabolism of lipids properties [8-10]. The inclusive bioactivities indicate that GA contains different chemotherapeutic agents. Although several phytochemistry works have been

reported on the pods, flowers, leaves, heartwood, bark, and roots of *A. senegal*, rare investigations have been made into the gum. Flavone, catechin, polyphenols, tannins, chalcones, alkaloids, and flavonoids have been identified in GA extracts [11].

A few studies have been reported on the antimicrobial activity of GA, including against several human pathogenic bacterial and fungi [5], inhibition activity against three food rotten microorganisms [8], against twelve different microorganisms [9], and cariogenic oral bacteria [10]. On the other hand, no insecticidal or larvicidal activities have been reported in GA extracts.

Currently, using phytochemicals for health, food, agricultural, and other needs has been preferred over synthetic substances. This tendency has resulted from some adverse effects raised from using chemical agents; mainly drug side effects, poisonousness to human, toxicity extended to beneficial organisms, drug resistance, environmental pollution, non-biodegradable nature, etc. To exploit the biologically active compound from plants, Sasidharan [12] determined the foremost stages to be conducted, namely extraction, pharmacological screening, isolation and characterization of bioactive compound, and toxicological and clinical evaluation. Therefore, the aim of this study was to screen the phytochemical content and the bioactivity of GA aqueous extract in terms of antimicrobial and mosquito larvicides.

Materials and methods

Reagents/chemicals materials

All the chemicals and reagents used in this study were of analytical grade. Dimethyl sulfoxide (DMSO), acetic anhydride, sulfuric acid, gelatin salt, and ferric chloride (purity 95 - 99.87 %) were obtained from Sigma-Aldrich (Germany). Other chemicals, such as aluminum chloride and potassium hydroxide, were from SDFCL (India). Purified distilled water was prepared in the laboratory. All glassware was from Marienfeld (Germany) and Exax (USA), and filter paper and discs were from Whatman. An oven, water bath, and incubator were from Nüve, Turkey.

Collection of gum material and extraction

The gum Arabic (GA) was collected in October 2016 from *Acacia senegal* var. *senegal* trees naturally growing in Abkrusholla, South Kordofan State, Sudan. The collected GA was crunched into ground powder and stored in containers until use. About 30 g of gum material was macerated in 100 mL distilled water until being dissolved with the assistance of a magnetic stirrer, then left at ambient temperatures (25 - 30 °C) for 24 h for more hydration. The extract was filtered with Whatman filter paper No. 41 and evaporated under room temperature. All extracts were stored at 4 °C until use in the experiments.

Phytochemical analysis

The phytochemical analysis of GA extract was assessed using standard protocols of analysis [2,12,13]. Qualitative detections were performed to determine the presence of different constituents individually, as described below. For each test, 1.0 mL of the gum extract was placed in a petri dish, dried in a water bath, and the obtained weight used for the phytochemical analyses.

The test for alkaloids was carried out by dissolving 3.0 gm of extract in 10 mL HCl 2 % or NH₄OH 10 % in three test-tube for each; then, Drognadroff's, Wagner's, and Mayer's reagents were added separately. For tannin analysis, 2.0 g of extract was dissolved in 10 mL ethanol, half of the solution used to test with ferric chloride, and half with gelatin salt. Triterpenes and sterols were tested as 1.0 g of extract dissolved in 6 mL of chloroform, and then a few drops of sulfuric acid added; the upper green layer indicated the presence of sterol, and the lower red brown ring indicated triterpenes. The Saponin test was performed by dissolving 0.5 g of extract in 10 mL of distilled water, vigorously shaking it for 30 seconds, allowing it to stand, and then observing it for the formation of foam. For cardiac glycosides, 0.1 g of extract was added to 1 mL glacial acetic acid anhydride, containing one drop of ferric chloride plus 1 mL sulfuric acid. Coumarin content was analyzed in 1.0 g extract that was dissolved in 10 mL distilled water in a test tube, and a filter paper saturated with vapor resulting from adding drops of 0.5 N KOH was

inspected under UV light. The Anthraquinone test was performed by dissolving 0.1 g extract in 1.0 mL distilled water, adding 5.0 mL of chloroform, shaking for 5 min, and adding 1.0 mL of ammonia solution (10 %) per 1.0 mL of chloroform fraction. The presence of flavonoids was determined by adding 1.0 g extract to 10 mL of ethanol, then transferring into four tests tube, and adding either NaOH, magnesium plus one drop of H₂SO₄ in water bath, aluminium chloride 1 % in methanol plus concentrated HCl, or ammonium solution.

Antimicrobial activity

Test organisms

Microorganisms used in the present study included four bacteria: two Gram (+) *Bacillus subtilis* (NCTC 8236), *Staphylococcus aureus* (ATCC 25923), and two Gram (-) *Escherichia coli* (ATCC 25922), *Pseudomonas arginosa* (ATCC 27853), and one fungi *Candida albicans* (ATCC 7596).

The aseptically cultured microorganisms were washed off with normal saline to produce a suspension of 10¹⁰ CFU/mL. The stock suspensions were diluted, and 0.02 mL were used as inoculum in the experiments. The average number of developed colonies in each drop (0.02 mL) was counted after incubation [14]. The suspension was maintained on desired media agar slants, stored at 4 °C, and subcultured periodically.

Bioassay

The cup-plate agar diffusion method [15] was adopted with some minor modifications to assess the antimicrobial activity of the gum extract. One mL of the standardized bacterial stock suspension 10⁸-10⁹ CFU/ mL was thoroughly mixed with 100 mL of molten sterile nutrient agar, which was maintained at 45 °C. Aliquots of 20 mL nutrient (sabouraud dextrose for fungi) agar were used and were distributed into sterile Petri-dishes. The agars were left to set, and in each of these plates 4 cups (10 mm in diameter) were cut using a sterile cork borer (No. 4) and agar discs were removed. The cups were filled with 0.1 mL of the extract and allowed to diffuse at room temperature for two hours. Three extract concentrations of 2.5, 5 and 10 mg/mL were used for the determination of antimicrobial activity. Ciprofloxacin (antibacterial) and ketoconazole (antifungal) in a concentration of 5 mg/mL were used as a positive control, and DMSO was used as a negative control. The plates were then incubated at 37 °C for 18 h (for bacteria), or at 25 °C for 48 h (for fungi). After incubation, the diameters of the resultant growth inhibition zones were measured.

Larval bioassay

The larvicidal activity of the prepared gum water extracts at different concentrations was determined using larvae of *Culex quinquefasciatus* assay, according to the WHO [16] method. The eggs of *Culex* were collected from rainy season swamps in Kadrow area, Khartoum North, Sudan. The collected eggs were transferred into open petri dishes contained distilled water with powdered bread. The eggs were incubated for 4 - 6 days at ambient conditions (28±2 °C, 70±5 %RH, and 12 h light) until arriving at the larval phase. The third instar larvae of *Culex* were identified according to the morphological characteristics of common *C. quinquefasciatus* larvae in Sudan [17]. Stock solution of larvicide was prepared by adding 50 mg of the extract into 5 mL of DMSO. The larvicidal activity of extract was carried against larvae of *Culex* at the concentrations 0.0, 5.0, 50, and 500 µL. The control concentration consisted of 2 mL DMSO. Thirty larvae were released in 200 mL water and treated with the desired concentration. Dead larvae were taken after 24 h and 48 h of incubation, and results on mortality percentage were calculated using formula (1). The larvae were considered dead when they were incapable of moving even after being inspired to with a needle or when water was disturbed. The LC₅₀ (lethal concentration that kills 50 % of the exposed larvae) and LC₉₅ (lethal concentration that kills 95 % of the exposed larvae) values were calculated based on mortality data using probit analysis [18].

$$\text{Mortality (\%)} = \frac{\text{Number of death larvae}}{\text{Number of larvae introduced}} \times 100 \quad (1)$$

Statistical analysis

All experiments were conducted in triplicate. One-way analysis of variance (ANOVA) was carried out using genstat procedure [19]. The means separation was performed using Duncan's multiple range test at 5 % significance. The results were presented as mean and standard error (mean ± SE).

Results and discussion

The extractive relative percentage yield GA using water was found to be 11.23 % w/w, with the physical appearance colorless and gummy. The extractive value indicated the presence of polar secondary metabolites. This was an approximate measure of the chemical constituents present in the gum. Also, it assisted in determination of the adulteration and impurities of the plant derived drug.

Phytochemical screening of GA

The result, in **Table 1**, showed higher amounts of alkaloids and saponin, moderate amounts of cardiac glycosides, and trace amounts of tannins. Flavonoids, sterol/triterpene, anthraquinone, and coumarin were not detected in the studied sample. This agreed partly with Marwah *et al.* [11], which reported that GA contains alkaloids and tannins. However, both studies reported that GA contained other secondary metabolites, such as flavone, catechin, chalcones, and flavonoids, which were not detected herein. Inversely, Evans *et al.* [2] stated that a 10 % aqueous solution of GA gives no reaction for tannin with ferric chloride if of pharmacopoeial quality.

Other plant parts of *A. senegal* have been screened for secondary metabolites contents. The bark of *A. senegal* revealed the presence of tannins, saponins, and sterols, while alkaloids, glycosides, and flavonoids were not detected [20]. In contrast, alkaloids, glycosides, and flavonoids have been detected in *A. senegal* stem [21]. Abdel-Farid *et al.* [22] studied the metabolic profiling of the leave, flower, and pod parts of three *Acacia* species, viz. *A. nilotica*, *A. seyal*, and *A. laeta*. The results showed that plant parts, as well as species, affected the presence and the concentration of the phytochemicals studied.

Table 1 Phytochemical screening of secondary metabolites in gum Arabic extract

Phytochemical	Tests used	Quantity index*
Alkaloids (10 %NH ₄ OH)	Drogndroff	-
	Wagner	-
	Mayer	-
Alkaloids (HCl methanolic)	Drogndroff	+++
	Wagner	+++
	Mayer	+++
Flavonoids	NaOH	-
	AlCl ₃	-
	Mg/H ₂ SO ₄	-
	NH ₄ OH	-
Tannins	Ferric Chloride	+
	Gelatin	+
Saponins		+++
Sterols and Triterpenes		-
Coumarin		-
Cardiac Glycosides		++
Anthraquinone		-

*The quantity of the phytochemical is estimated by using the intensity of the test color as indicator; (+++) high, (++) moderate, (+) trace amounts, (-) not detected.

In general, the bioactive compounds were created by plants for protection against bacteria, i.e., phytoprotectants, and are responsible for the antimicrobial activity of GA [5]. Alkaloids have been shown to be effective anti-feeding against herbivorous pests, including insects and mammals. Also, alkaloids have toxic effects against some fungi and bacteria strains. The evidence for this role is increasing. Saponins are very useful in the treatment of upper respiratory tract inflammation and are reported to have anti-diabetic and anti-fungal properties. Cardiac glycosides are highly toxic plant secondary compounds. They are inhibitors of the plasma membrane Na⁺/K⁺-ATPase that are used for the treatment of heart failure. Also, cardiac glycosides are responsible for the poisoning of livestock. Tannins possess physiological astringents and are therefore used for treating intestinal disorders such as diarrhea and dysentery, and wound healing and ameliorate-inflamed mucus membrane. The typical effect of tannins is the inhibition of cell protein synthesis resultant from irreversible complex reaction with the proline-rich protein [23].

Antimicrobial activity

The crude extracts of Arabic gum at concentrations of 2.5, 5, and 10 mg/mL were subjected to antimicrobial assays by using the Cup plate method, and the inhibition zones were measured (mm) against four bacterial organisms (two gram positive; *B. subtilis*, *S. aureus* and two gram negative; *E. coli*, *P. arginosa*) and one fungal organisms (*C. albicans*); mean activities are shown in **Figure 1**.

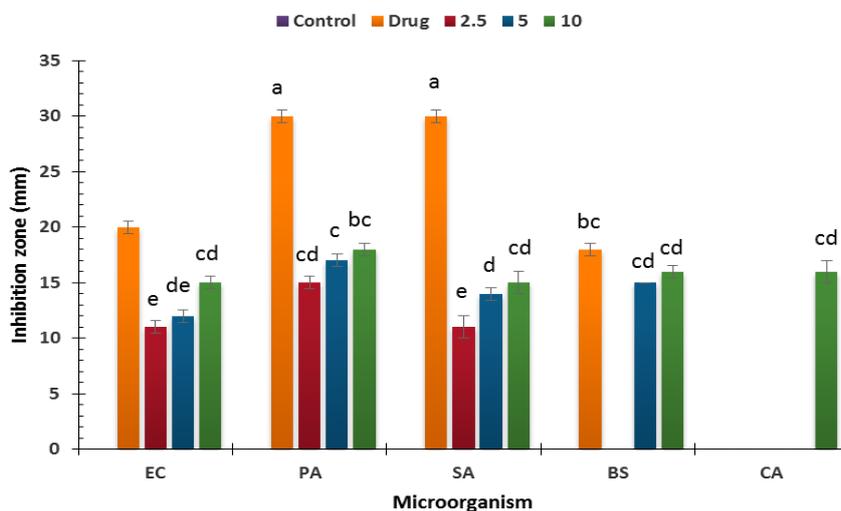


Figure 1 Antimicrobial activity of GA extract in different concentrations 0.0, 2.5, 5, and 10 mg/mL and standard antibiotics (ciprofloxacin and ketoconazole). The activity was expressed in terms of diameter of zone of growth inhibition in mm. Vertical bars represent standard errors. EC: *Escherichia coli*, PA: *Pseudomonas arginosa*, SA: *Staphylococcus aureus*, BS: *Bacillus subtilis*, CA: *Candida albicans*, GA: gum Arabic.

The aqueous extract of GA showed variable inhibitory effects, depending on the concentration and the microorganism type. Only the concentration 10 mg/mL showed effectiveness against all the microorganisms. The concentrations 2.5 and 5 mg/mL had no significant effect against the fungi *C. albicans* (**Figure 1**). Also, 2.5 mg/mL had no significant effect against *B. subtilis* (**Figure 1**). For clarity of the results, the size of diameter of the inhibition zone was expressed as < 9 mm: inactive, 9 - 12 mm: partially active, 13 - 18 mm: active, and > 18 mm: very active. Accordingly, the extract at concentration

10 mg/mL was active against *E. coli*, *S. aureus*, *B. subtilis*, and *C. albicans*, and very active against *P. aeruginosa*.

Similarly, GA showed a moderated effect against *P. aeruginosa*, while *Micrococcus luteus* and *B. subtilis* were unaffected by the presence of GA [8]. Alawi *et al.* [5] studied antimicrobial activity using different organic fractions of GA from two sources against *S. aureus*, *E. coli*, and *Klebsiella pneumonia*. The Sudanese GA showed higher antimicrobial activity against all bacterial strains. Similar to the present study, water extracts showed moderate activity against all bacterial strains. In another study, GA exhibited a moderate inhibition activity against *Streptococcus mutans* [10]. Furthermore, Bnuyan *et al.* [9] compared the antimicrobial activities of aqueous extracts of commercial gum samples of *A. senegal* and *A. syeal* against eleven bacterial strains and one fungus. The results revealed that GA (*A. senegal*) was highly active against only *S. epidermidis*, *St. pneumoniae*, *K. pneumoniae*, *Serratia spp.*, and *C. albicans*, while *A. syeal* displayed superior activity against all the tested microorganisms. On the other hand, gum from other *Acacia* showed growth inhibitory activities, such as *A. arabica*, on *Prophyromonas gingivalis* and *Prevotella intermedia* [24].

The antimicrobial effects of GA may be due to the presence of high amounts of alkaloids and saponins that were detected in the extract. Extracts of different parts of *A. senegal* also exhibited antimicrobial activities. Various organic solvent extracts of *A. senegal* root-heartwood revealed significant activity against *E. coli*, *S. aureus*, and *C. albicans* [3]. Hexane extract of the bark showed activity against *S. aureus* and the fungus *C. albicans*, while methanol extract showed activity against *E. coli*, *B. cereus*, and fungi *C. albicans* and *A. niger* [6].

Larvicidal activity

Investigation of the larvicidal activity of GA (*Acacia senegal* gum) was carried out for the first time. The preliminary experiment (**Table 2**) concluded that *C. quinquefasciatus* was sensitive to GA extract. However, the same value of 86.7 % mortality occurred by the maximum concentration (500 µL) within 24 h and 48 h. The effect was dose dependent and significantly different ($P < 0.05$) between concentrations and from the control in all cases. Increasing the exposure period resulted in a progressive increase in mortality by the lower concentrations (5, 50 µL). Likewise, the smallest values of LC₅₀ and LC₉₅ were obtained in 48 h (**Table 3**), indicating that increasing the exposure time enhanced the larvicidal activities compared to 24 h. A similar effect of exposure times was reported by Kamaraj *et al.* [25], where *A. concinna* seed extracts nearly doubled the mortality in *C. quinquefasciatus* larvae after 48 h.

Table 2 Larvicidal effect of GA extract on *Culx quinquefasciatus* 3rd instar larvae after 24 and 48 h

Extract (µL)	Number of larvae	Parentage mortality (mean ±SE)	
		24 h	48 h
0	30	0.0±0.0 ^d	0.0±0.0 ^d
5	30	13.33±0.2 ^c	14.44±2.9 ^c
50	30	66.67±1.9 ^b	73.33±5.1 ^{ab}
500	30	86.67±1.0 ^a	86.67±1.9 ^a

Different letters within the column indicate statistically significant differences between means according to Duncan's multiple range test ($P < 0.05$). GA: gum Arabic, SE: standard errors.

Table 3 Lethal dose of GA aqueous extract against *Culex quinquefasciatus* 3rd instar larvae after 24 and 48 h

Exposure period	LC ₅₀ µL (LC ₅₀ Log)	LC ₉₅ µL (LC ₉₅ Log)	Regression equation	r ²
24 h	32.74 (1.515)	653.75 (2.8154)	$y = 34.605x - 2.4274$	0.9612
48 h	28.58 (1.4561)	545.77 (2.737)	$y = 35.131x - 1.1538$	0.9363

LC₅₀: lethal concentration that kills 50 % of the exposed larvae, LC₉₅: lethal concentration that kills 95% of the exposed larvae, r²: regression coefficient.

This larvicidal effect reported in the present study may be attributed to high viscosity characterized GA, which inhibits the movement and uptake of nutrients by larvae. Additionally, the existence of high amounts of acidic alkaloid saponin, cardiac glycosides, and tannins has a toxic effect on the larvae.

To the best of knowledge, no larvicidal activities of gums from any *Acacia* species have been reported. However, extracts of different parts of various *Acacias* exhibited varied activities against *C. quinquefasciatus*. Compared with those previous studies, GA extracts in the present study showed medium values of 32.74 µL (LC₅₀) and 653.75 µL (LC₉₅), as essential oil from seed of *A. nilotica* showed 5.23 mg/L (LC₅₀) and 9.71 mg/L (LC₉₀) [26], and acetone extract from leaf of *A. ferruginea* showed 5362.6 ppm (LC₅₀) and 5630.2 ppm (LC₉₀) [27]. Insecticidal effects of plant extracts varied not only according to mosquito species, geographical varieties, or plant species and parts used, but also due to extraction methodology and the polarity of the solvents used [28].

Generally, the dissimilarities in the bioactivities of GA can be accredited to variations in the sources of *A. senegal* gums. The principal gum origin variables, including age of the trees, climatic conditions, and soil environment, greatly affected the gum constitutes and structure [29]. A simple immunological analysis of 16 gum exudates obtained from 13 *Acacia* species displayed that each of the gum samples studied had a unique composition. Even more, gum obtained from different sub-species of a single species (e.g., *A. nilotica* has four sup-species) presented very distinct chemical compositions [7].

Conclusions

Gum Arabic (*A. senegal*) extract was obtained using water as a solvent and had good effect on bacteria, fungi, and larvae. More research is needed on GA to determine the active component that generates antimicrobial and larvicidal activities. Further work on purification of gum extracts to isolate the bioactive metabolites and their structures must be elucidated.

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