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Biological study of some Gelditsia species

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Abstract: The present study investigates the antimicrobial, anti-inflammatory effects of the total methanolic extracts and different fractions of *Gelditsia triacanthos* fruits and *Gelditsia caspica* leaves and anti-mycobacterial activity of total methanolic extract and different fractions of *Gelditsia triacanthos* fruits. The antimicrobial activities were investigated against G +ve and G –ve bacteria and fungi. Methanolic extract of *G. caspica* had prominent antifungal activity against *A. niger* about 88% of ketoconazole potency. Butanol fraction of *G. caspica* had relative antimicrobial activity approximately 64% of Gentamicin standard against *S. typhimurium*. Butanol fraction of *G. triacanthos* had moderate antifungal activity against *A. niger* approximately 73% of ketoconazole potency. Petroleum ether extract of *G. triacanthos* achieved antifungal activity approximately 75% of ketoconazole potency against *C. Albicans* whereas it had relative antimicrobial activity approximately 70% of Gentamicin standard against *S. typhimurium*. While anti-inflammatory properties of the total methanolic extracts and different fractions of *G. triacanthos* fruits and *G. caspica* leaves revealed that the ethyl acetate fraction of *G. caspica* has anti-inflammatory activity about 29% of the standard diclofenac, whereas different fractions of *G. caspica* were found to be less effective as anti-inflammatory. Finally total methanolic extracts and different fractions of *G. triacanthos* fruits were evaluated for their anti-tuberculosis activity and showed that Butanol fraction achieved anti-mycobacterial potency about 0.006% of the standard isoniazid.

Keywords: *Gelditsia triacanthos*; *Gelditsia caspica*; antimicrobial; anti-inflammatory; anti-tuberculosis.

1. INTRODUCTION

With 36 tribes, 727 genera, and 19,327 species, the Fabaceae (or Leguminosae) bean family is the third biggest flowering plant family after Orchidaceae and Asteraceae[1]. *Gleditsia*, the Locust tree, is a Fabaceae genus that includes roughly 14 species of deciduous trees[2]. The majority of *Gleditsia* species diversity is found in Eastern Asia, Africa, North, and South America[3]. In the flora of Egypt, *Gelditisia caspica* Desf, and *Gelditisia triacanthos.* L. are perennial shrubs grown mainly for ornamental purposes[4]. In oriental traditional medicine genus *Gleditsia* is used as an expectorant and diuretic[5]. They are employed in treating skin disorders, carbuncles, apoplexy, productive cough, headaches, asthma, and expectorant[6]. The crude extracts and refined molecules of the Genus *Gleditsia* contain a wide spectrum of biological actions, according to pharmacological research, such as reduction of inflammation, analgesic[7], cytotoxic[6], antiallergic[8], antihyperlipidemic[9], antimicrobial[10], antioxidant[11] and antimutagenic activities[12]. As a result, it was important to conduct this biological study in order to shed light on this species that grows in Egypt. This research was undertaken to evaluate antimicrobial and anti-inflammatory activities of different extracts of *G. caspica and G. triacanthos* and anti-tuberculosis activity of different extracts of *G. triacanthos.*

2. METHODS

2.1. Plant collection and drying

Fresh leaves of *G. caspica* Desf. were obtained from Al-zohriya Garden in Egypt during April 2018. While *G. triacanthos* fruits were gathered from Giza Zoo Public Garden, Egypt in April 2018. Mrs.

Therese L. Youssef, head expert for plant identification at El-Orman Public Garden in Giza, Egypt, validated plant identification. A voucher specimen (Reg. No. GT-2018, GS-2018 respectively) of plants was deposited within Pharmacognosy Department herbarium.

2.2. Preparation of plant extract and different fractions

Plant material was dried in a well aerated shaded place and powdered. Air-dried powder (50g) of each plant was separately extracted via maceration using 70% methanol (3x500ml) at 25±2 ºC for 1 day each maceration. Solvent was removed after the extract had been filtered using a rotary evaporator (Buchi Co., Switzerland) at 50 °C to obtain semi-dried extract which suspended in distilled water (50 ml) and filtered. The water-soluble portion was partitioned with petroleum ether (60-80 ºC) then ethyl acetate and *n*-butanol.

2.3. Antimicrobial study

2.3.1. Materials

All the extracts and fractions were assessed for their antimicrobial effectiveness against standard strains of microorganisms in vitro. Tested microorganisms and standard drug mentioned in Table1&2. DMSO was used as solvent for tested samples, the extracts and fractions were at a concentration of 1 mg/ml.

2.3.2. Method of testing

Agar well diffusion method as mentioned in (Balouiri *et al*., 2016)[13] was used to assess the antimicrobial activity of the samples.

This experiment was done in triplicate, and the zones of inhibition were quantified in millimetres.

2.4. Anti-inflammatory study

Human monocytes U937 were used to study the effect of samples on histamine release. Diclofenac sodium (Sigma), at a various concentration (7.8, 15.6, 31.2, 62.5, 125, 250,500 and 1000 µg/ml) was used as a positive control. Results were given as a percentage of inhibition and IC_{50}, has been determined by (Venkata *et al*., 2012)[14].

2.5. Anti-mycobacterial activity

Standard cultures of *mycobacterial tuberculosis* were procured from American Type Culture Collection. *M. tuberculosis* was grown in medium, and conditions reported by (Lu *et al*., 2011; Elsayed *et al.*, 2021)[15,16]. Isoniazid was used as a control (Sigma), at various concentrations (0.24, 0.48, 0.98, 1.95, 3.9, 7.8, 15.6, 31.2, 62.5 and 125 *µg*/ml). MIC against *M. tuberculosis* was determined by the MABA. The MIC was determined as the lowest concentration that caused a 100% decrease in fluorescence relative to the replicate bacterium-only control's mean.

3. RESULTS

3.1 Antimicrobial activity

Antimicrobial properties of 70% methanolic extract and different fractions of *G. caspica* and *G. triacanthos* were examined individually as shown in Table 1 and 2, results were expressed as zone of inhibition.

Table 1. Antimicrobial properties of aqueous methanolic extract and different fractions of *G. caspica*.

Sample code / Tested microorganisms	Inhibition zone (mm)				
	70% MeOH extract	Pet. ether fraction	EtOAc fraction	*n*-BuOH fraction	Control
Bacteria classified as Positive Gram:					Gentamycin
***Staphylococcus aureus* (RCMB010010)**	11	10	13	9	24
***Bacillus subtilis* (RCMB 015)**	10	NA	NA	NA	26
***Staphylococcus epidermidis* (RCMB 009)**	NA				28
Bacteria classified as Negative Gram:					Gentamycin
***Escherichia coli* (RCMB 010052)**	NA	NA	10	NA	30
***Proteus vulgaris* (RCMB 004)**	NA				25
***Salmonella typhimurium* (RCMB 006)**	8	10	NA	11	17
Fungi					Ketoconazole
***Aspergillus niger* (RCMB 002005)**	NA				15
***Aspergillus fumigatus* (RCMB 002008)**	15	13	NA	NA	17
***Candida albicans* (RCMB 005003)**	NA				20

*NA: No activity

Table 2. Antimicrobial properties of aqueous methanolic extract and different fractions of *G. tricanthos*.

Sample code / **Tested microorganisms**	**Inhibition zone (mm)**				
	70% MeOH extract	Pet. ether fraction	EtOAc fraction	n-BuOH fraction	Control
Bacteria classified as Positive Gram:					Gentamycin
***Staphylococcus aureus* (RCMB010010)**	13	11	12	12	24
***Bacillus subtilis* (RCMB 015)**	11	10	NA	9	26
***Staphylococcus epidermidis* (RCMB**		NA			28
Bacteria classified as Negative Gram:					Gentamycin
***Escherichia coli* (RCMB 010052)**	NA	NA	NA	9	30
***Proteus vulgaris* (RCMB 004)**		NA			25
***Salmonella typhimurium* (RCMB 006)**	11	12	NA	9	17
Fungi					Ketoconazole
***Aspergillus niger* (RCMB 002005)**	NA	NA	NA	11	15
***Aspergillus fumigatus* (RCMB 002008)**		NA			17
***Candida albicans* (RCMB 005003)**	NA	15	NA	NA	20

*NA: No activity

3.2. Anti-inflammatory activity

The Anti-inflammatory activity of different extracts of *G. triacanthos* and *G. caspica* was evaluated using histamine release inhibitory percent (Table 3). Diclofenac was used as a positive control. Results were given as IC_{50}.

Table 3. IC_{50} (µg/ml) of different extracts of *G. triacanthos*, *G. caspica* and diclofenac.

***G.* species**	**IC_{50} (µg/ml)**				
	70% Methanolic	**Pet. ether**	**Ethyl acetate**	***n*-Butanol**	**Diclofenac**
G.	5446.1	7210.3	905.4	96.04	17.94
G. caspica	948.6	7926.3	60.93	377.9	17.94

3.3. Anti-mycobacterial activity

Anti-mycobacterial activity of different extracts of *G. triacanthos* by the (MABA) method and isoniazid was used as standard. The results are displayed in Table 4.

Table 4. MIC and MIC_{90} of different extracts of *G. triacanthos* and isoniazid.

G.	**70% Methanolic**	**Pet. ether**	**Ethyl acetate**	***n*-Butanol**	**Isoniazid**
MIC_{90}	>125	>125	>125	44.99	0.4
MIC	>125	>125	>125	62.5	0.24

4. DISCUSSION

4.1. Antimicrobial

The search for antimicrobials from natural sources has received much attention and efforts have been put in to identify compounds that can act as suitable antimicrobials agent to replace synthetic ones, with less toxic and more effective medicines in controlling the growth of microorganism[17]. Antimicrobial properties of the methanolic extract, fraction extracted by petroleum ether, fraction extracted by ethyl acetate and fraction extracted by butanol from *Gleditsia caspica* as shown in Table1 revealed that the Ethyl acetate fraction of *G. caspica* was the most potent against *S. aureus* with the largest zone of inhibition (13 mm) among the tested samples, followed by methanolic extract, with zone of inhibition 11 mm, both fractions had antimicrobial potency about 50% of Gentamycin standard which achieved zone of inhibition 24 mm. Butanol fraction of *G. caspica* had relative antimicrobial activity approximately 64% of Gentamicin against *S. typhimurium* with zone of inhibition 11 mm, then pet. ether fraction, with inhibition zone 10 mm. Methanolic extract and pet. ether fraction from *G. caspica* were potent in contrast to *A. fumigates* with zone of inhibition 15 mm and 13 mm, respectively. Methanolic extract of *G. caspica* had prominent antifungal activity as it showed 88% potency when compared with ketoconazole which achieved zone of inhibition 17 mm.

Table 2 revealed that methanolic extract of *G. triacanthos* was the most potent against *S. aureus* with the largest zone of inhibition 13 mm among the tested samples, followed by ethyl acetate and butanol, with zone of inhibition 12 mm, both fractions had relative antimicrobial activity when compared with Gentamycin as a standard which achieved zone of inhibition 24mm. Pet. ether fraction of *G. triacanthos* was the most potent against *S. typhimurium* with the largest zone of inhibition 12 mm, among the tested samples, followed by methanolic extract, with zone of inhibition 11 mm, they had 60% antimicrobial activity of Gentamicin standard which achieved zone of inhibition 17 mm. Pet. ether fraction of *G. triacanthos* had relative antimicrobial activity approximately 70% of Gentamicin standard. Butanol fraction of *G. triacanthos* only has activity against *A. niger* among tested extracts with zone of inhibition 11 mm. It had antifungal activity approximately 73% of ketoconazole potency, which achieved zone of inhibition 15 mm. Pet. ether fraction of *G. triacanthos* only has activity *C. albicans* among tested extracts with zone of inhibition 15 mm. It had antifungal activity approximately 75% of ketoconazole potency, with zone of inhibition 20 mm.

4.2. Anti-inflammatory activity

Although a substantial progress in medicinal research have been made during the past decade, there is still a crucial need for discovering new drugs to treat inflammation diseases which represent one of the world's health problems[18]. Inflammation is a reaction that occurs when living tissues are damaged. The inflammatory response is a protective mechanism that develops in response to greater inflammation caused by injury to living tissues. Histamine is a vasoactive amine that generated during inflammation and plays a critical function in the initial inflammatory response [18]. The Anti-inflammatory activity of different extracts of *G. triacanthos* and *G. caspica* was evaluated using histamine release inhibitory percent. Diclofenac was used as a positive control. The tested four extracts of *G. triacanthos* possessed variable anti-inflammatory activity in the order: *n*-butanol fraction > fraction of ethyl acetate > methanolic extract > pet. ether fraction. According to the IC_{50}; *n*-butanol fraction had anti-inflammatory activity about 18.6% of the standard diclofenac, while Ethyl acetate fraction had about 0.02%. Finally pet. ether extract and methanolic extract achieved anti-inflammatory potency less than 0.003% of the standard diclofenac. The tested four extracts *G. caspica* possessed variable anti-inflammatory activity in the order: ethyl acetate fraction > *n*-butanol fraction > methanolic extract > pet. ether fraction: ethyl acetate fraction has anti-inflammatory activity about 29% of the standard diclofenac, while *n*-butanol fraction and methanolic extract had potency about 0.04% and 0.01% respectively. Finally pet. extract achieved anti-inflammatory potency less than 0.002% of the standard diclofenac. The relative anti-inflammatory potential of ethyl acetate fraction could be attributed to its phenolic content[20]

4.3. Anti-tuberculosis activity

Tuberculosis (TB) is a potentially fatal infectious illness that mostly affects the lungs. The germs that cause TB move from person to person by

small droplets discharged into the air by coughs and sneezes. The lungs are the most affected, although it can affect any organ of the body, including the stomach (abdomen), glands, bones, and neurological system[21]. Screening of Anti-mycobacterial activity of different extracts of G. *triacanthos* individually was carried out by the microplate alamar blue assay (MABA) method[15] and isoniazid was used as standard. According to MIC_{90} tabulated in Table 4; *n*-butanol fraction has anti-mycobacterial activity with MIC_{90} and MIC 62.5 and 44.99, respectively; standard isoniazid showed MIC_{90} and MIC at 0.4 and 0.24, respectively. Finally, *n*-butanol fraction achieved anti-mycobacterial potency about 0.006 % of the standard isoniazid.

5. CONCLUSIONS

The hunt for medications derived from natural sources has gotten a lot of attention and effort to identify compounds that to replace synthetic ones, for disease management. Total extracts of *G. triacanthos* fruits and *G.caspica* leaves and their fractions showed promising results as anti-microbial agent against some bacteria and fungi, further *in vitro* studies should be done against other different strains. The relative anti-inflammatory potential of ethyl acetate fraction could be attributed to its phenolic contents. Methanolic extract of *G. triacantho*s and different fractions had not significant results as anti-tuberculosis. Thus, further phytochemical study should be done to isolate pure compounds for clinical studies.

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List of Abbreviations: IC_{50}: Concentration of the sample under the test conditions, block 50% of its activity;
MIC: The lowest concentration effecting a reduction in fluorescence of 100% relative to the mean of replicate bacterium-only controls;
MIC_{90}: The lowest concentration effecting a reduction in fluorescence of 90% relative to the mean of replicate bacterium-only controls;
MABA: Microplate Alamar Blue Assay;
Pet. ether: Petroleum ether (60-80 ºC).

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