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Research Article
Evaluation of Anti-microbial Activity of *Ex vitro* and Callus Extracts from *Commiphora gileadensis*

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Abstract

Background and Objective: *Commiphora gileadensis* a medicinal plant rare species. A large amount of plant materials were needed to produce secondary metabolite under *in vitro* culture. Therefore, callus is used in the *in vitro* culture, since it can proliferate quickly and continuously provide an appropriate amount of plant which used for extracting the antimicrobial compounds from *C. gileadensis*. **Materials and Methods:** Rapid protocol for optimum callus production has been assessed to overcome limitations of the conventional propagation methods. The effect of plant growth regulator (PGR) on the regeneration of *C. gileadensis* was investigated for callus induction experiment using a standard MS medium with various concentrations of 6-Benzyl adenine (BA), Kinetin (Kn), 2,4-dichlorophenoxy acetic acid (2,4-D) and naphthalene acetic acid (NAA) at 0.0, 0.5, 1.0,1.5, 2.0 and 2.5 mg L^{-1}. **Results:** The result showed that the maximum regeneration of callus induced the fresh and dry weight were obtained 5675±1321 and 376.7±56.9 mg, respectively on MS media containing 2 mg L^{-1} 2,4D + 0.5 mg L^{-1} BA after 12 weeks. The anti-bacterial and anti-fungal activities of *C. gileadensis* were evaluated using the callus and *ex vitro* extracts, six bacterial species fungal genera were used the agar well diffusion method used of 25, 50, 75 and 100 µL methanolic or ethonlic extracts of *ex vitro* and callus had considerable inhibition effects on the tested bacteria and fungi. **Conclusion:** Callus culture technique may be an important tool to get the *C. gileadensis* quickly as compared to the natural growth phenomenon where it takes many years. Moreover, it's give us an opportunity to get the active constituent without destroying the plant available in nature. The results of the present study can improve our understanding of the economic importance of *C. gileadensis* as activity ingredient antimicrobial agent and provided methods for its preparation.

Key words: *Commiphora gileadensis*, anti-bacterial, anti-fungal, callus induction, *ex vitro* extracts, plant growth regulator

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Competing Interest: The author has declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Arabic books from the Middle Ages provide information regarding balsam, a small tree of the Burseraceae family, called *Commiphora gileadensis* (L.) C. Chr. (or *Commiphora opobalsamum*)[1,2]. The *C. gileadensis* L. growing in Saudi Arabia, it has a unique climate, influenced by the Mediterranean as a moderating feature and the desert as a drying factor[3]. It is also recognized locally as balsam and well recognized for the luxurious perfume produced from it, in addition to the special medicinal characteristic that was attributed to its seeds, bark, wood and sap. It was known in early times as a perfume and incense plant, generally, established in particular ecological areas[4].

Moreover, investigations of the antimicrobial activity of *C. gileadensis* showed the plant sap inhibitory effect against *Bacillus cereus* and the blocking of *Pseudomonas aeruginosa* lectins[5]. On other hand, Al-Sum *et al.*[6] in his study showed that the antimicrobial activity of basham (*C. gileadensis* L.) aqueous extracts against two Gram-negative bacteria *Escerichia coli*, *Salmonella typhi* and fungi *Aspergillus niger*, *Penicilium italicum* to inhibit radial growth of both fungi. Moreover, mycelial dry and fresh weights of both fungi were reduced significantly by the extracts of *C. gileadensis*. According to Abbas *et al.*[7], different extracts and several of the isolated compounds and various extracts were assessed for different biological activities in some several of the isolated compounds and various extracts were assessed for different biological activities in some *in vitro* assays. Initial screening for phytochemical of the vegetative portions of *C. opobalsamum* showing the existence of volatile bases, triterpenes, sterols, volatile oil, flavonoids and saponins[8].

Seeds of *C. gileadensis* are rarely found in nature for normal agricultural propagation, for that, it is necessary to search for alternative methods for its efficient propagation. One of these methods could be plant tissue culture. The formation or induction of *in vitro* callus can be achieved by using a special combination of plant growth regulators, mostly high auxin to cytokinin ratio depending on the genotype and the endogenous hormone content[9,10]. As a starting material for callus induction, almost all kinds of tissues and organs can be used. Callus is an undifferentiated mass of tissue which appears on explants after transfer onto growth medium with suitable plant hormones[11]. Different growth hormones are used to promote callus induction and development[12].

Callus can be used for obtaining virus free plant, mutagenic studies or as source of protoplasts and suspension cultures and it is mostly used for production of secondary metabolites[9,12]. On the other hand, *in vitro* plant regeneration from a leaf of *Artemisia vulgaris* obtained callus in MS medium supplemented with 1.0 mg L^{-1} BAP and 3.0 mg L^{-1} NAA[13]. On the other hand, callus on *Artemisia annua* were initiated on Murashige and Skoog[14] medium (MS) supplemented with sucrose (30 g L^{-1}), myoinositol (100 mg L^{-1}), medium were supplemented with naphthalene acetic acid (NAA), indole-acetic acid (IAA), indole-butyric acid (IBA) and 2,4-dichlorophenoxyacetic acid[15]. While, callus initiated from micro-propagated *A. absinthium* plantlets on (MS) basal medium supplemented with different concentrations of BAP, IAA kinetin, 2,4-D and NAA individually or in combination[16].

There is an urge to discovery naturally producing substances from plants with anti-fungal semisynthetic products or antimicrobial activity as an alternative to antibiotics. The objective of the current study was to develop simple reliable protocol for efficient callus induction and to study antimicrobial activity of *ex vitro* and callus extracted from *Commiphora gileadensis* plants.

MATERIALS AND METHODS

Plant materials and culture conditions: Total time required to conduct this study about 11 months from February-December, 2016. Acquisition and analysis of data, drafting of article and revision January-October, 2017. Tissue culture plants were obtained from Biology Department, College of Sciences in Yanbu, Taibah University, plants and Seeds of *C. gileadensis* were obtained and collected from Yanbu district in Saudi Arabia from its natural habitats. *In vitro* seeds were cultured on (MS) medium Murashige and Skoog[14] supplemented with and 3% sucrose. The pH was adjusted to 5.8 and 8 g L^{-1} agar was dissolved using microwave prior to autoclaving. In each of 250 mL flask, 60 mL of medium was dispensed. The breather hole of each container was plugged with aluminum fuel and media was autoclaved for 20 min at 121°C. Micro-shoots were incubated at 24±2°C with a 16 h photo-period and photo-synthetic photon flux density (PPFD) of 50 μmol m^{-2} sec^{-1} supplied by cool white florescent lamps. Micro-hoots produced were subcultured every 6 weeks onto hormone free MS medium and subculture every 5-7 weeks to generate sufficient plant material. This method was achieved according to Davey and Anthony[17].

Effect of medium type on *in vitro* grown plantlets: Micro-shoots, 2-3 mm in length were subcultured on MS medium supplemented with 2 mg L^{-1} 2,4D+ 0.5 mg L^{-1} BA

from the previous experiment. The effect of different media was examined as follows: Full strength MS[14], WPM[18], AP[19], AN[20], NN[21] and B5[22].

Each treatment consisted of 30 replicates and each replicate contained four micro-shoots. Data were collected regarding the number of new shoots per initial micro-shoot, maximum shoot height and callus diameter after 12 weeks in culture.

Growth media and callus induction: Pieces of micro-shoots (2-3 mm) were placed into 9 cm sterile Petri dishes, 15 mL of MS[14], incubated in dark in a growth room at 24±2°C, which were supplemented with different concentrations of 6-Benzyladenine (BA), Kinetin (Kn) as an auxin and 2,4-dichlorophenoxyacetic acid (2,4-D), naphthalene acetic acid (NAA) as cytokine at 0.0, 0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1}. A combinations of Auxin/Cytokine concentrations that give the best callus induction was test.

Micro-shoots were placed on the surface of sterile solid media. Each treatment contained 10 completely randomized replicates. Data were recorded after 12 weeks of incubation for callus induction percentage, texture and color. Callus fresh weight was recorded for 3 randomly selected replicates. Callus samples were dried to a constant weight at 70°C and dry weights were recorded.

Test organisms: bacterial and fungal species: Six bacterial species were used in this study: About 4 g positive bacteria (*Staphylococcus aureus*, *Bacillus cereus, Micrococcus latus* and *Staphylococcus epidermidis*) and two Gram-negative bacteria (*Escherichia coli* and *Salmonella typhimurium*).

Six fungal species were also included in the current study as follows: *Aspergillus nidulans*, *Aspergillus niger mutant black, Penicillium italicum*, *Penicillium chrysogenum*, *Phytophthora infestans* Location 1 and *Phytophthora infestans* Location 2. All microbial species were obtained from the Microbiology Laboratory, Biology Department, College of Sciences in Yanbu, Taibah University. Fungal and bacterial strains were cultured on Potato Dextrose Agar (PDA, Himedia, India) and nutrient agar (NA, Fluka, Germany), respectively.

Preparation of plant extracts: Twenty grams of *Commiphora gileadensis* plants materials (*ex vitro* and callus) were dried in lab room for 2 weeks, ground to a fine powder in liquid nitrogen (LN) and then extracted with 100 mL methanol or 100 mL absolute ethanol by soaking for 1 week[23]. The solvents were removed using rotary evaporator (eppendorf, Germany) under reduced pressure at temperatures below 50°C. Stock solutions of extracts were prepared in dimethyl sulphoxide (DMSO)[24] in 250 µg μL^{-1} concentration and then different volumes (25, 50, 75 and 100 µL) were tested against microbes. Control experiments were performed using both positive (bactericide (oxytetracycline) and fungicide (cyclohexamine) and DMSO serves as negative controls. Extracts were dissolved in DMSO and evaluated for their ability to inhibit the bacterial and fungal growth.

Antimicrobial activity assay by the agar well diffusion method: Agar well technique was used to investigate the antimicrobial activity of *C. gileadensis*. Aliquots extract (25, 50, 75 and 100 µL) at 250 µg μL^{-1} concentration were added into wells and left for 1 h to diffuse. The plates were then incubated at 37°C for 24 h. Oxytetracycline and Cyclohexamine was used as positive control and prepared in 250 µg μL^{-1} for anti-bacterial and anti-fungal activity. Dimethylesulfoxide (DMSO) was tested as negative control. The microbial growth was determined by measuring the diameter of the inhibition zone and was compared with positive control. Each treatment consisted of three replicates and each replicate contained three Petri dishes. This method was achieved according to Perez *et al.*[25] and Nakamura *et al.*[26].

Statistical analysis: The experiments were designed as completely randomized design. Data were subjected to one way ANOVA analysis. Mean values were compared according to Duncan Multiple Range test at $p = 0.05$. Data were analyzed using SPSS (20) package.

RESULTS

Effect of medium type on *in vitro* grown plantlets: The optimal shoots/explant was recorded on MS medium, whereas lowest numbers of shoots/explant were recorded on WPM cultures (Table 1). In addition, medium composition affected length of shoots. Maximum shoot lengths were obtained when explants were grown on MS medium (Table 1). Shoot growth on other base media showed significant reductions in the developed shoots/explants, compared with those on full strength MS medium. On other hand, the plants that grew on MS medium showed the best quantity and creamy colors of callus.

Callus induction and growth: To find out the optimum medium for callus growth and development, fresh weight of callus was evaluated after 12 weeks of growth periods. Calli

Table 1: Effect of different medium type on number of shoot, shoot length, callus formation of *in vitro* grown *C. gileadensis* after 12 weeks growth periods on medium supplemented with 2, 2,4D+0.5 mg L^{-1} BA

Media type	Number of axillary shoot/explant	Shoot length (mm)	Callus presence
AP	4.41±0.86^{bc}	17.41±1.94^{b}	++
WPM	2.90±0.46^{a}	13.62±1.30^{a}	+
AN	3.89±0.51^{bc}	14.40±1.08^{a}	+
MS	7.67±0.97^{d}	21.87±2.02^{c}	+++
NN	3.77±0.63^{bc}	15.19±0.77^{a}	++
B5	5.22±0.85^{bc}	18.33±1.21^{b}	+++

Means followed by the same letter within each column are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four micro-shoots. Values are the means±standard error. Callus column, -: No callus, +: <5 mm in diameter, ++: <10 mm in diameter and +++: >10 mm in diameter

Table 2: Effect of different concentrations of BA on callus formation, fresh weight and dry weight of *in vitro* grown *C. gileadensis* after 12 weeks growth periods

Concentration (mg L^{-1})	Fresh weight (mg)	Dry weight (mg)
0.0	868±225^{a}	72.71±8.90^{a}
0.5 mg L^{-1} BA	4166±1563^{b}	237.3±174.4^{b}
1 mg L^{-1} BA	4201±967^{b}	251.5±42.99^{b}
1.5 mg L^{-1} BA	4552±531^{b}	256.3±35.3^{b}
2 mg L^{-1} BA	4893±384^{b}	291.9±66.7^{b}
2.5 mg L^{-1} BA	5003±448^{b}	302.2±71.2^{b}

Means followed by the same letter within each column are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four micro-shoots. Values are the means±standard error

Table 3: Effect of different concentrations of Kn on callus formation, fresh weight and dry weight of *in vitro* grown *C. gileadensis* after 12 weeks growth periods

Concentration (mg L^{-1})	Fresh weight (mg)	Dry weight (mg)
0.0	898±266.0^{a}	83.1±8.90^{a}
0.5 mg L^{-1} Kn	2722±389.0^{b}	161.2±35.4^{bc}
1 mg L^{-1} Kn	3547±329.0^{b}	197.6±44.29^{bc}
1.5 mg L^{-1} Kn	4113±487.0^{c}	243.4±61.23^{bc}
2 mg L^{-1} Kn	4287±967.1^{c}	263.3±82.09^{bc}
2.5 mg L^{-1} Kn	4384±987.0^{c}	277.5±81.96^{c}

Means followed by the same letter within each column are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four micro-shoots. Values are the means±standard error

Table 4: Effect of different concentrations of 2,4D on callus formation, fresh weight and dry weight of *in vitro* grown *C. gileadensis* after 12 weeks growth periods

Concentration (mg L^{-1})	Fresh weight (mg)	Dry weight (mg)
0.0	837±278^{a}	78.63±18.5^{a}
0.5 mg L^{-1} 2,4D	2280±433^{b}	131.10±37.2^{a}
1 mg L^{-1} 2,4D	4641±922^{c}	269.60±72.7^{b}
1.5 mg L^{-1} 2,4D	4922±657^{c}	259.50±41.7^{b}
2 mg L^{-1} 2,4D	5639±873^{c}	338.20±54.3^{b}
2.5 mg L^{-1} 2,4D	5746±1002^{c}	321.70±61.4^{b}

Means followed by the same letter within each column are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four micro-shoots. Values are the means±standard error

were initiated from shoot tips on MS media supplemented with different concentration of BA, 2,4-D, Kn or NAA at 0.5, 1.0, 1.5, 2.0, 2.5 mg L^{-1} (Table 2- 5). Results showed

Table 5: Effect of different concentrations of NAA on callus formation, fresh weight and dry weight of *in vitro* grown *C. gileadensis* after 12 weeks growth periods

Concentration (mg L^{-1})	Fresh weight (mg)	Dry weight (mg)
0.0	866±251.0^{a}	74.7±12.70^{a}
0.5 mg L^{-1} NAA	2655±154.3^{b}	181.7±55.76^{b}
1 mg L^{-1} NAA	3154±971.2^{bc}	247.8±48.50^{bc}
1.5 mg L^{-1} NAA	3411±1028.3^{c}	294.3±66.10^{c}
2 mg L^{-1} NAA	4119±567.1^{c}	307.9±84.90^{c}
2.5 mg L^{-1} NAA	4336±877.6^{c}	319.3±74.40^{c}

Means followed by the same letter within each column are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four micro-shoots. Values are the means±standard error

Table 6: Effect of combination of different concentrations 2,4D and BA on callus formation, fresh weight and dry weight of *in vitro* grown *C. gileadensis* after 12 weeks growth periods

Concentration (mg L^{-1})	Fresh weight (mg)	Dry weight (mg)
0.0	913±244^{a}	77.9±19.11^{a}
2 mg L^{-1} 2,4D+0.5 mg L^{-1} BA	5675±1321^{d}	376.7±56.9^{d}
2 mg L^{-1} 2,4D+1 mg L^{-1} BA	2651±604.92^{c}	153.6±42.55^{bc}
2 mg L^{-1} 2,4D+1.5 mg L^{-1} BA	2743±368.3^{bc}	164.7±31.71^{bc}
2 mg L^{-1} 2,4D+2 mg L^{-1} BA	2860±297.1^{bc}	158.7±44.3^{bc}
2.5 mg L^{-1} 2,4D+0.5 mg L^{-1} BA	2203±242^{bc}	141.4±41.6^{b}
2.5 mg L^{-1} 2,4D+1 mg L^{-1} BA	1779±277^{b}	111.3±34.3^{a}
2.5 mg L^{-1} 2,4D+1.5 mg L^{-1} BA	1809±293^{b}	116.2±29.8^{a}
2.5 mg L^{-1} 2,4D+2 mg L^{-1} BA	1449±261^{ab}	93.8±44.3^{a}

Means followed by the same letter within each column are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of 16 replicates and each sample contained four micro-shoots. Values are the means±standard error

significant differences between the four media of either BA, 2,4-D, Kn or NAA pertaining to their effect on callus growth.

Initially small yellowish calli developed on the cut ends within 10 days of growth and subsequently covered the entire disc of the explants with BA, 2,4-D, Kn or NAA (Table 2-5). Maximum callus fresh weight was obtained on MS medium enriched with 2.5 mg L^{-1} BA, 2.5 mg L^{-1} 2,4D, 2.5 mg L^{-1} NAA or 2.5 mg L^{-1} Kn concentrations (Table 2-5). Of the various concentrations studied, the callus induced in medium supplemented with Kn or NAA was dark brown.

The MS media supplemented with mixed of different concentrations of BA (0.5, 1.0, 1.5, 2.0 and 2.5 mg L^{-1}) and 2,4D (2 or 2.5 mg L^{-1}) (Table 6), lower callus fresh

Table 7: Anti-bacterial activity of methanolic plant extract of *C. gileadensis*

		Inhibition zone (mm) in different solvent system			
		Methanol		Control	
Strain	Crude amount (µL)	*Ex vitro*	Callus	Positive oxytetracycline	Negative DMSO
Staphylococcus aureus	25	11.2± 0.49^{a}	6.50±0.33^{a}	25.6±0.62^{c}	0^{a}
	50	15.4±0.51^{d}	10.30±0.58^{b}	36.2±0.74^{e}	0^{a}
	75	19.8±0.57^{f}	13.90±0.51^{de}	38.4±0.90^{1e}	0^{a}
	100	24.0±0.54^{k}	17.60±0.58^{j}	41.8±0.84^{f}	0^{a}
Micrococcus luteus	25	12.6±0.52^{b}	8.20±0.38^{b}	24.3±0.78^{c}	0^{a}
	50	18.0±0.64^{e}	9.80±0.40^{c}	37.1±1.15^{e}	0^{a}
	75	20.7±0.68^{j}	10.20±0.52^{c}	39.1±0.90^{e}	0^{a}
	100	23.8±0.68^{k}	11.00±0.52^{c}	43.6±1.45^{f}	0^{a}
Escherichia coli	25	12.5±0.44^{b}	18.60±0.60^{j}	32.1±0.72^{d}	0^{a}
	50	16.1±0.56^{d}	23.40±0.68^{k}	37.7±0.58^{e}	0^{a}
	75	17.6±0.54^{e}	24.10±0.62^{k}	37.4±0.74^{e}	0^{a}
	100	19.8±0.58^{f}	26.90±0.74^{l}	43.2±1.15^{f}	0^{a}
Bacillus cereus	25	10.6±0.55^{a}	19.30±0.54^{h}	15.9±0.79^{a}	0^{a}
	50	15.7±0.58^{d}	24.60±0.60^{k}	19.8±0.83^{a}	0^{a}
	75	16.6±0.41^{d}	26.70±0.64^{l}	27.3±0.76^{c}	0^{a}
	100	18.0±0.66^{e}	27.90±0.52^{l}	43.6±1.11^{f}	0^{a}
Staphylococcus epidermidis	25	12.3±0.44^{b}	12.80±0.43^{d}	26.7±0.98^{c}	0^{a}
	50	19.4±0.59^{f}	16.10±0. 49^{f}	36.1±1.20^{e}	0^{a}
	75	21.4±0.56^{j}	17.50±0.51^{j}	40.4±1.23^{f}	0^{a}
	100	25.7±0.48^{d}	19.60±0.63^{h}	45.3±1.52^{j}	0^{a}
Salmonella typhimurium	25	14.1±0.38^{c}	8.90±0.42^{a}	16.1±1.20^{a}	0^{a}
	50	19.7±0.60^{f}	10.60±0.44^{c}	23.7±0.88^{b}	0^{a}
	75	21.1±0.62^{j}	12.10±0.48^{d}	36.3±1.30^{e}	0^{a}
	100	24.3±0.72^{k}	13.90±0.50^{de}	41.73±0.88^{f}	0^{a}

Mean followed by the same letter within each species are not significantly different according to LSD test at $p \leq 0.05$. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means±standard error. Data obtained after incubation 24 h on NA media

(1449±261 mg) and dry weight (93.8±44.3 mg) were recorded on MS medium supplemented with 2.5 mg L^{-1} 2,4D+ 2 mg L^{-1} BA. However, using MS medium combination of 2 mg L^{-1} 2,4D and 0.5 mg L^{-1} BA concentrations resulted in soft, friable yellowish calli and maximum callus fresh and dry weight were 5675±1321 and 376.7±56.9, respectively, compared to other media. The result found to be effective for callus induction in *C. gileadensis*, it was induced after 12 weeks and increased the production significantly (Table 6).

Anti-bacterial methanolic extract activity: The results showed that 25, 50, 75 and 100 µL methanolic extracts of *ex vitro* and callus had inhibitory effect on 6 tested bacterial species as represented in Table 7, methanolic extracts from *ex vitro* and callus differ significantly in their activities against the tested bacteria. The maximum inhibition zone of *ex vitro* plants was 24.3±0.72 against *Salmonella typhimurium* (Table 7). Moreover, callus extracts showed different degree of inhibitory activity, the maximum inhibitory activity rate was 27.9±0.52 against *B. cereus*. The lowest sensitivity to callus methanolic extract was *S. typhimurium* and *M. luteus* (Table 7).

Anti-bacterial ethanolic extract activity: The results in Table 8 showed that *Staphylococcus aureus* was the most sensitive bacterial species to *ex vitro* ethanolic extracts. The ethanolic extracts from *ex vitro* and callus showed different significantly in their activities against the tested bacterial strains. Callus ethanolic extract shown different degree of anti-bacterial activity, it was the less sensitive to *Micrococcus luteus* and *Salmonella typhimurium*.

Anti-fungal activity

Anti-fungal methanolic extracts activity: The results in Table 9 showed that *ex vitro* methanolic extract had inhibitory activity against all fungal spices except *Aspergillus nidulans* compared with callus extract while callus methanolic extract showed less sensitivity against *Penicillium chrysogenum*. On the other hand, the maximum inhibition zone with methanolic callus extract was found against *Phytophthor ainfestans* (L:1). The activity of methanolic extract of callus and *ex vitro* was not significantly different in anti-fungal activity. It showed the highest anti-fungal activity on *Phytophthora infestans* (L:1) with 42.3±2.10 mm zone of inhibition.

Table 8: Anti-bacterial activity of ethanolic plant types extract of *C. gileadensis*

		Inhibition zone (mm) in different solvent system			
		Methanol		Control	
Strain	Crude amount (µL)	*Ex vitro*	Callus	Positive oxytetracycline	Negative DMSO
Staphylococcus aureus	25	11.6±0.4[a]	8.30±0.34[a]	29.7±0.61[c]	0[a]
	50	15.2±0.44[b]	14.30±0.82[b]	35.1±0.92[d]	0[a]
	75	16.7±0.46[b]	17.30±0.52[c]	38.3±1.10[d]	0[a]
	100	19.1±0.54[b]	21.90±0.60[d]	42.1±1.2.88[f]	0[a]
Micrococcus luteus	25	15.3±0.42[b]	3.30±0.12[a]	18.6±0.78[a]	0[a]
	50	19.7±0.58[b]	5.10±0.22[a]	24.7±1.20[b]	0[a]
	75	21.5±0.48[bc]	6.30±0.18[a]	37.3±1.40[d]	0[a]
	100	23.7±0.67[cd]	8.80±0.33[a]	44.3±1.38[f]	0[a]
Escherichia coli	25	9.8±0.38[a]	9.30±0.44[a]	28.2±0.62[c]	0[a]
	50	16.2±0.52[b]	13.00±0.38[a]	37.0±0.70[d]	0[a]
	75	17.9±0.58[b]	14.90±0.42[b]	38.6±0.82[df]	0[a]
	100	20.1±0.84[b]	17.20±0.78[c]	41.7±1.00[f]	0[a]
Bacillus cereus	25	12.0±0.32[a]	9.30±0.28[a]	14.3±0.36[a]	0[a]
	50	19.1±0.33[c]	14.00±0.68[b]	20.1±0.92[a]	0[a]
	75	21.0±0.52[c]	17.20±0.56[c]	29.3±0.68[c]	0[a]
	100	23.2±0.62[cd]	18.60±0.32[c]	36.0±1.36[d]	0[a]
Staphylococcus epidermidis	25	11.2±0.32[a]	5.60±0.22[a]	26.2±0.48[b]	0[a]
	50	18.3±0.68[c]	7.20±0.30[a]	36.7±0.90[d]	0[a]
	75	19.6±0.72[c]	8.80±0.28[a]	39.2±1.10[df]	0[a]
	100	24.1±0.68[cd]	10.20±0.38[ab]	45.1±1.538[g]	0[a]
Salmonella typhimurium	25	9.3±0.22[a]	4.40±0.20[a]	25.2±0.42[b]	0[a]
	50	17.3±0.33[bc]	5.20±0.26[a]	29.7±0.92[c]	0[a]
	75	18.3±0.62[bc]	6.40±0.32[a]	37.3±0.86[d]	0[a]
	100	23.3±0.38[d]	8.00±0.42[a]	43.8±1.20[g]	0[a]

Mean followed by the same letter within each spices are not significantly different according to LSD test at $p \leq 0.05$. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means±standard error. Data obtained after incubation 24 h on NA media

Table 9: Anti-fungal activity of methanolic plant extract of *C. gileadensis*

		Inhibition zone (mm) in different solvent system			
		Methanol		Control	
Strain	Crude amount (µL)	*Ex vitro*	Callus	Positive oxytetracycline	Negative DMSO
Penicillium Italicum	25	14.9±0.36[bc]	16.2±0.42[c]	22.3±0.44[b]	0[a]
	50	17.0±0.88[c]	20.8±0.82[d]	27.5±0.84[c]	0[a]
	75	24.2±0.92[de]	23.1±0.48[de]	32.6±0.52[d]	0[a]
	100	27.0±1.15[ef]	25.7±0.50[e]	36.0±0.72[f]	0[a]
Penicillium chrysogenum	25	21.3±0.44[d]	5.2±0.12[a]	29.9±0.62[c]	0[a]
	50	24.7±0.74[d]	8.0±0.22[a]	34.1±0.72[df]	0[a]
	75	28.1±0.66[f]	9.6±0.36[a]	36.70±1.2[df]	0[a]
	100	33.3±1.20[j]	11.1±0.30[b]	43.7±1.40[g]	0[a]
Aspergillus nidulans	25	13.3±0.32[b]	16.4±0.42[c]	26.3±0.56[c]	0[a]
	50	16.1±1.15[bc]	20.4±0.44[d]	34.6±0.72[df]	0[a]
	75	18.3±0.66[c]	22.3±0.66[d]	38.5±0.44[f]	0[a]
	100	21.4±0.56[de]	24.6±0.66[e]	44.8±1.30[g]	0[a]
Phytophthora infestans (L:1)	25	22.3±.76[de]	16.7±0.42[c]	28.0±0.48[c]	0[a]
	50	29.8±0.48[e]	18.9±0.42[c]	31.0±0.66[d]	0[a]
	75	36.7±0.92[k]	24.1±0.48[d]	38.9±1.30[f]	0[a]
	100	42.3±2.10[l]	32.6±1.30[f]	45.7±1.32[k]	0[a]
Phytophthora infestans (L:2)	25	14.4±0.34[b]	6.3±0.25[a]	13.9±0.28[a]	0[a]
	50	18.1±0.84[c]	8.7±0.22[a]	19.6±0.44[cd]	0[a]
	75	21.7±0.68[d]	11.4±0.32[b]	21.8±0.66[c]	0[a]
	100	24.6±1.6[e]	14.2±0.88[b]	27.5±0.92[e]	0[a]
Aspergillus niger mutant. Black	25	9.6±0.28[a]	6.7±0.18[a]	21.8±0.38[b]	0[a]
	50	12.7±1.4[a]	8.4±0.40[a]	29.4±1.60[c]	0[a]
	75	18.3±0.70[c]	11.7±0.36[b]	36.3±1.6d[f]	0[a]
	100	27.0±1.3[d]	18.9±0.80[c]	43.7±1.80[k]	0[a]

Means followed by the same letter within each spices are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means±standard error. L: 1 represents location one. L: 2 represent location two. Data obtained after incubation 48 h on PDA media

Table 10: Anti-fungal activity of ethanolic plant extract of *C. gileadensis*

		Inhibition zone (mm) in different solvent system			
		Methanol		Control	
Strain	Crude amount (µL)	*Ex vitro*	Callus	Positive oxytetracycline	Negative DMSO
Penicillium Italicum	25	13.7±0.42^{b}	15.3±0.28^{b}	19.3±0.60^{a}	0^{a}
	50	16.1±0.46^{c}	18.4±0.44^{c}	28.0±0.56^{b}	0^{a}
	75	18.6±0.42^{c}	20.8±0.52^{cd}	32.1±0.82^{c}	0^{a}
	100	21.4±1.3^{de}	24.0±1.20^{d}	37.7±1.50^{d}	0^{a}
Penicillium chrysogenum	25	15.2±0.42^{c}	5.3±0.18^{a}	26.1±0.46^{b}	0^{a}
	50	18.0±0.48^{c}	7.6±0.28^{a}	33.6±0.72^{c}	0^{a}
	75	21.9±0.62^{de}	9.9±0.34^{a}	39.5±0.82^{d}	0^{a}
	100	23.6±0.66^{e}	12.4±0.32^{b}	44.4±1.10^{f}	0^{a}
Aspergillus nidulans	25	13.8±0.26^{b}	19.6±0.70^{c}	29.5±0.86^{b}	0^{a}
	50	17.4±0.78^{c}	25.2±1.20^{d}	33.4±0.94^{c}	0^{a}
	75	19.6±0.50^{c}	29.5±0.48^{d}	37.3±0.88^{d}	0^{a}
	100	21.8±0.66^{de}	33.7±0.78^{e}	45.0±1.32^{f}	0^{a}
Phytophthora infestans (L:1)	25	22.3±0.62^{de}	9.5±0.22^{a}	14.3±0.42^{a}	0^{a}
	50	29.8±1.32^{f}	16.4±0.44^{b}	19.7±0.60^{b}	0^{a}
	75	31.7±0.76^{f}	19.5±0.72^{bc}	26.5±0.72^{b}	0^{a}
	100	33.2±1.30^{f}	22.7±1.40^{c}	38.7±1.12^{d}	0^{a}
Phytophthora infestans (L:2)	25	13.3±0.28^{b}	5.5±0.08^{a}	17.8±0.44^{a}	0^{a}
	50	17.1±0.86^{c}	8.3±0.34^{a}	23.6±0.70^{b}	0^{a}
	75	19.1±0.84^{c}	11.5±0.40^{b}	28.3±0.56^{b}	0^{a}
	100	21.6±0.76^{de}	14.3±0.88^{b}	29.8±1.36^{b}	0^{a}
Aspergillus niger mutant black	25	5.4±0.28^{a}	3.3±0.08^{a}	19.8±0.44^{a}	0^{a}
	50	9.6±0.66^{a}	4.9±0.20^{a}	26.3±0.90^{b}	0^{a}
	75	15.2±0.72^{b}	6.8±0.46^{a}	35.1±1.80^{c}	0^{a}
	100	23.2±1.80^{de}	7.4±0.22^{a}	43.6±1.52^{f}	0^{a}

Means followed by the same letter within each spices are not significantly different according to Duncan Multiple range test at $p \leq 0.05$. Each treatment consisted of three replicates and each sample contained three Petri dishes. Values are the means±standard error. L:1 represents location one. L:2 represents location two. Data obtained after incubation 48 h on PDA media

Anti-fungal ethanolic extracts activity: The results in Table 10 showed similarity between *ex vitro* and callus for the anti-fungal activities, the *ex vitro* extract exhibited the highest rates of anti-fungal activity except *Penicillium italicum* and *P. chrysogenum*, it was more effective in callus extract of anti-fungal activity. However, *Phytophthora infestans* (L:2) showed less susceptibility towards ethanol callus extracts. Moreover, *Phytophthora infestans* (L:1) showed the highest sensitivity (20.3 mm) to the ethanol extract of the *ex vitro* plant.

DISCUSSION

Comparative study of *in vitro C. gileadensis* plantlets grown on different medium includes MS, WPM, AP, NN, AN and B5 were studied. High shoot numbers were produced on MS medium[27-30]. The type of media significantly affected the number of *Prunus* species shoot/explants length independent of the culture media[31,32].

The best quantity and creamy colors of callus shown in the plants that growth on MS medium. The MS basal medium supported callus induction, subsequently shoot and root formation[33-35]. However, other media types were rarely reported such as B5[22,36-41] and Woody Plant Medium (WPM)[4,42-45]. Hustache *et al.*[46] accounted that the best mineral media for callus induction was the Knop and Ball (KB) medium.

In medium supplemented with 2,4-D or NAA callus were generated from the explants after 2 months in incubation media. It was soft and friable white from the different concentrations that tested, the maximum response was shown on MS medium supplemented with 2.0 mg L^{-1} NAA. The MS media was supplemented with 2.0 mg L^{-1} NAA shown the maximum fresh weight with the best color and callus texture after 2 months. Al-Ajlouni *et al.*[47] were able to initiate callus of *Hordeum vulgare* L. on MS media supplemented with 2,4-D. This result is in agreement with Abe and Futsuhara[48] study.

In the present results, the media containing combined of 2 mg L^{-1} 2,4D+0.5 mg L^{-1} BA always produced the largest callus size. Similar result were reported for *Arabidopsis*[49]. This suggested that the callus induction medium induces callus

through the genetic pathway mediating lateral root initiation and this callus is not as de-differentiated as previous thought[50,51]. However, the hormonal combination of 2,4-D and kinetin was previously found to be effective in producing optimum callus induction in *Aquilaria malaccensis* Lam, in which 70-73% of callus induction was recorded[49].

The maximum callus fresh weight was acquired in this study, on MS medium complemented with 2.5 mg L^{-1} 2,4-D and it was light yellowish and friable. Similar results was reported when study *Capparisspinosa* L. in which[52] 2,4-D. This result is identical to the earlier finding on *Oryza sativa*[48]. On other hand, present results shown that there were significant differences among NAA treatments in concern to callus growth. Results indicated that different explants and different hormones combines had significant difference in callus[53]. Moreover, the callus of cotyledons explants (in hormonal treatment of 2 mg L^{-1} BAP+0.5 mg L^{-1} NAA) with 98.66% had the major amount of survival and the minimum was achieved in media containing 0.5 mg L^{-1} NAA+0.5 mg L^{-1} ABA in abaxial explants[54].

Many health care specialists reported drug resistance due to frequent misuse of antibiotics. In this study, antimicrobial activity of *ex vitro* and callus of *C. gileadensis* plant was evaluated using ethanolic and methanolic extracts. The obtained results showed that ethanol and methanol extract revealed a wide anti-fungal and anti-bacterial activity. However, the present study, chemical compound was extracted by ethanol or methanol. Lemberkovics *et al.*[55] showed that the composition of essential oils in aromatic plants is significantly influenced by the technique of extraction, mostly the distribution of the chemical compound present in the plants. The study used *C. gileadensis* reported the extracts shown substantial inhibitory effects against different bacterial and fungal species with several degrees of growth inhibition[56]. In that study, the methanol extract was higher activity related to water extracts. However, the methanolic extracts of *C. gileadensis* displayed reasonable anti-bacterial activity, for the methanolic extract[56]. The crude of methanolic extract for *C. gileadensis* shown a considerable anti-mycobacterial activity and the minimum inhibitory concentration[54] was 62.5 µg mL^{-1}. The *C. gileadensis* extracted of using both organic solvents such as methanol, ethanol and acetone and hot water in the tested bacteria were multidrug resistant, *Escherichia coli*, *Micrococcus lutes*, *Klebsiella pneumoniae* and *Shigella sonnei* shown the lower activities recorded for the methanolic extract of *Commiphora gileadensis, Cymbopogon schoenanthus* and *Abutilon bidentatum* against all tested bacteria[57].

The green portion of *C. opobalsamum* used with ethyl acetate extract was reasonably active against *Plasmodium falciparum*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*[7]. However, the results study proved that the methanolic extract of bark of plant displayed an activity against *Candia species*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Results in this study demonstrated that *C. gileadensis* has a high potential as an antimicrobial medicinal plant. Plant extract containing active compound interfere with bacteria and fungi cell wall which has inhibitory mechanism on the growth of these micro-organism[58]. Furthermore, micro-organisms showed different susceptibility to chemical substances against various resistance fungal and bacterial strains[58]. This investigation therefore justifies the use of ethanolic and methanolic extract.

CONCLUSION

Due to the presence of many active compounds in *C. gileadensis,* the *ex vitro* and callus extracts in this study showed a broad-spectrum of activity against both Gram-positive and Gram-negative bacteria in addition to fungi. Furthermore, *C. gileadensis* could be used as a health remedy in folk medicine. Bioactive compounds from this plant can be utilized in the formulation of antimicrobial agents for a verity of bacterial and fungal infections treatment. Additionally, the callus can propagate quickly and continuously, it can provide sufficient source of plants for use especially for a rare species as *C. gileadensis*.

SIGNIFICANCE STATEMENT

This study discovered the method for producing callus from rare *C. gileadensis* plant that can be beneficial for providing the high amount of antimicrobial compounds extracted by the callus rather than the conventional plant. this study will assist researcher in development scheme through a genetic transformation because high frequency of plantlet regeneration in this technique raises the success potential of transformed plantlets.

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