

## ANTICANDIDAL ACTIVITY AND CHEMICALS OF *COMMIPHORA GILEADENSIS* (L.) C. CHR. (BURSERACEAE) GROWING IN SOUTH WESTERN REGION OF SAUDI ARABIA

MAHA AL-QARNI<sup>1</sup>, SULAIMAN ALRUMMAN AND MAHMOUD MOUSTAFA\*

*Department of Biology, College of Science, King Khalid University, Abha, Saudi Arabia*

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### Abstract

Anticandidal activities of stem extracts of *C. gileadensis* growing in various locations and chemicals had been evaluated. Methanol extract of fresh and dry solvent extracts in Tathleeth A region had the highest activity ( $2.2 \pm 0.3$  cm) and ( $1.7 \pm 0.2$  cm), respectively. Cyclohexanone of fresh stem extract and diethyl ether of dry extract showed inhibition activities ( $2.1 \pm 0.1$  cm) of Tathleeth B. The highest activity gained from fresh hot water extract ( $1.4 \pm 0.1$  cm) and from dry extract of dichloromethane ( $1.7 \pm 0.0$  cm) in Tabala C. The maximum inhibiting activity obtained from hot water extract ( $1.8 \pm 0.4$  cm) and from chloroform of dry extract ( $2.0 \pm 0.0$  cm) in Tabala D. GC-MS analysis showed that the dominant compound in Thatleeth A and Thatleeth B is Bisethylamine, Tabala C are 5-Methyl-2(5H)-furanone and palmitic acid and Tabala D is Amitrole. HPLC showed that Tabala D had the highest amount from B<sub>1</sub> and folic acid. Thus, plant's biochemical parameters are profoundly influenced by their locations.

### Introduction

Biological indicators of an individual's such as morphology, anatomy, physiology, behavior, biochemistry and molecular data could be influenced according to the specific plant locations (Moustafa *et al.* 2016). One of the important aromatic and as natural toothbrush plants growing in Sarawat Mountains, southwest part of Saudi Arabia, in Arabian Peninsula and in East African is *Commiphora gileadensis* (L.) C. Chr. belong, Burseraceae family. It had a historical reputation as an herbal remedy in Arabic culture, known by the name "Balm of Judea" and "Balsam" which means medicine (Majrashi 2013). According to the environment, the size of *C. gileadensis* can be ranged from small shrub to a tree of several meters and it has male and female flowers in different trees. The fruit of *C. gileadensis* is unique with dividing lines giving a fake shape of multiple lobes fruit, although it has only one seed. It grows up to an elevation of 1,200 m and it provides scrub cover on most dry stony hills up to about 1,500 m. Extract from leaves, flowers, bark, and seeds of *C. gileadensis* are used in many medicinal purposes in traditional medicine. The inner side of the bark is used as an antiseptic for treating the wounds, and the juice of ground bark as an anti-allergic medicine of the inflamed skin. Local tribes in Oman have also used the plant extract to treat rabies, and as a bath for cleaning of newborns. The plant extracts showed an antimicrobial activity against many bacterial and fungal species (Iluz *et al.* 2010).

The Sarawat Mountains are located in an area of high elevation, where there is a greater likelihood of cloud cover, precipitation, snowfall, and stronger winds. Each of these factors has the capacity to influence how living things act biologically in specific environments. Therefore, the goal of this study is to ascertain how the plant's specific location have an impact on anti-candidal activities produced by solvent extracts of *C. gileadensis* and its chemical contents.

\*Author for correspondence: <mfmostfa@kku.edu.sa>. <sup>1</sup>Department of Biology, Faculty of Science, University of Bisha, P.O. Box 551, Bisha-61922, Saudi Arabia.

### Materials and Methods

Healthy plant sample had been collected from four locations in Bisha area, Saudi Arabia at different altitude above the sea levels as followings: Tathleeth A; 19° 58' 42.96" N, 42° 16' 2.28" E at height 1402.52 m. Tathleeth B; 19° 46' 39.36" N, 42° 44' 5.28" E at 1372.51 m in height. Tabala C; 19° 58' 42.24" N, 42° 13' 36.12" E at 1435.66 m in height. Tabala D; 19°, 58' and 42.96" N, 42° 16' and 2.28" E at 1416.38 m in height (Fig. 1). From every location, 249 gms of fresh stems of *C. gileadensis* plants were collected.

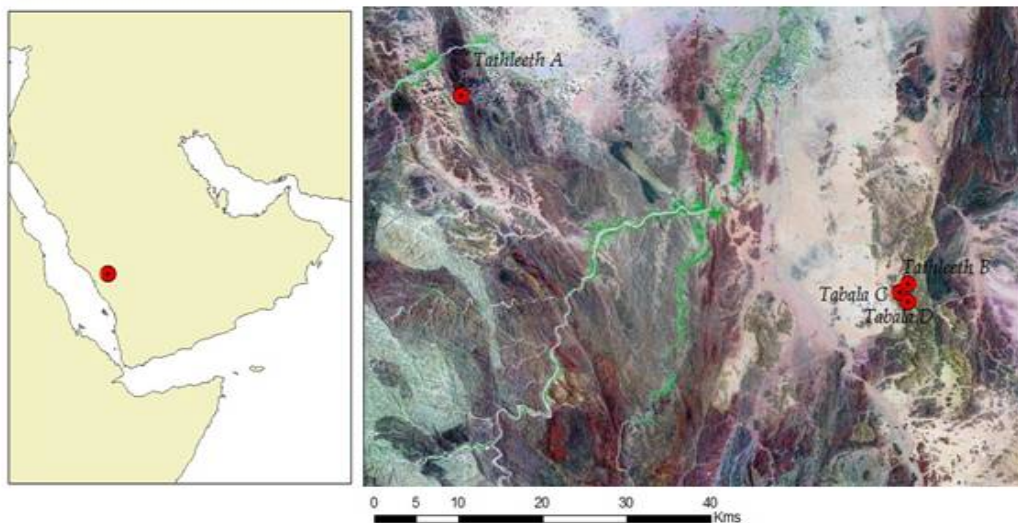


Fig.1. Sites map of collected samples from Tathleeth A, Tathleeth B, Tabala C and Tabala D in Saudi Arabia

Three gms of stems of *C. gileadensis* either dry or fresh were added to 7 ml of methanol, acetone, hot water, chloroform, dichloromethane, diethyl ether and cyclohexane. Each sample with solvent was kept in rotary shaker at 100 rpm at 25°C for five days. Solvents were evaporated from each sample using oven adjusted at 49°C then the resultant extract was weighted and kept in incubator at 4°C for further use. All the extract was dissolved in the 3 ml of sterile dimethyl sulfoxide (DMSO) and kept at 4°C for anticandidal activity. A single colony from previously prepared *C. albicans* was inoculated into nutrient broth and grown at 30°C overnight with shaking at 180 rpm. Activated cells of *C. albicans* for inoculation were prepared (Moustafa *et al.* 2020). Anti-candidal inhibition activity of each solvent against *C. albicans* were examined by well-agar diffusion method (Moustafa *et al.* 2013; Moustafa and Alrumman 2015). Twenty mL from Mueller-Hinton sterile agar was poured in sterile Petri dishes plate and remained for one hour in 24°C. One millilitre from freshly prepared *C. albicans* was spread equally using sterilized loop. A circle hole in four mm in diameter was made using a sterilized cork-borer and then 100 ul of the plant extract added to each hole. To allow the plant extract for fully diffuse into agar all Petri dishes were kept at room temperature at 23°C for one hour. Cefoxitin-30 mcg was used as positive control while dimethyl sulfoxide (DMSO) used as a negative control under the same condition. All plates containing *C. albicans* isolate were incubated at 29°C for 48 hrs. The sensitivities of the *C. gileadensis* solvent extracts were assessed by measuring the diameter of inhibition zone around the hole. Data analyzed using one-way ANOVA with Turkey post hoc test (Graphpad Prism Version 6 software, UK).

Gas chromatography-mass spectrometry analysis (GC-MS) was used to examine the chemical components in a *C. gileadensis* stem extract. Three grams of dried *C. gileadensis* stems were soaked in three mL of 100% alcohol (Sigma-Aldrich) for twelve hours and filtered through Whatmann filter paper No. 41 with two grams of sodium sulphate. The filter paper was wetted with absolute alcohol before filtering and filtrate was concentrated and its volume reduced by bubbling nitrogen gas into the solution. The extract, which contains both polar and non-polar phytochemicals were subjected to analysis and the chemicals were identified using the database of National Institute Standard and Technology (NIST).

B<sub>1</sub>, B<sub>2</sub>, B<sub>12</sub> and folic acid vitamins had been determined by using a Shimadzu model HPLC system (Shimadzu Corporation, Kyoto, Japan). Shimadzu HPLC system composed of a solvent delivery module (LC-10AD) integrated with a double plunger reciprocating pump, ultraviolet-visible spectrophotometry (UV-Vis) detector (SPA-10A), column oven (CTO-10A) and 20- $\mu$ l injection loop have been used. Potassium dihydrogen phosphate (KH<sub>2</sub>PO<sub>4</sub>), formic acid, methanol, acetic acid and HCL were used for running the HPLC system. Standard folic acid, vitamins B<sub>1</sub>, B<sub>2</sub> and B<sub>12</sub> were purchased from Sigma-Aldrich, Buchs SG, Switzerland. 15.07g of prepared *C. gileadensis* stem extract powder and 10 ml of 0.1N HCl and 80 ml of distilled water were added, and the mixture was then refluxed over a boiling water bath for 15 minutes. In a volumetric flask, distilled water (DW) was used to cool the flask and the volume to 100 ml was adjusted. The content in the flask was centrifuged and filtered with 0.45  $\mu$ m membrane filter paper before injection to the system in order to remove the suspended material. As a mobile phase a gradient from 30~70 (in eight minutes) from methanol and potassium dihydrogen phosphate (0.05 M, pH 4.2, adjusted with formic acid) was used. The flow rate was adjusted at 1 ml min<sup>-1</sup> and detection wave length at 254 nm with an injection volume of 20  $\mu$ l (Perveen *et al.* 2009).

## Results and Discussion

*In vitro* efficacy of anticandidal activities of stem extracts of *C. gileadensis* from four various locations in south western part of Saudi Arabia is shown in Fig. 2. clearly showed that there is a significant variation in the anticandidal inhibition properties of the extracts due to solvent type applied, plant locations, type of plant extract either fresh or dry. Negative control dimethyl sulfoxide (DMSO) did not show any effect on *C. albicans* growth while Cefoxitin-30 mcg that used as a positive control produced a clear inhibition zone.

In Tathleeth A region (Fig. 2A) all solvents extract displayed a range of anticandidal inhibition activities. It showed that methanol fresh extract had the highest inhibiting activity with a zone of inhibition (2.2 $\pm$ 0.3cm), followed by dichloromethane (1.5  $\pm$  0.1 cm) and diethyl ether had the least activity (0.5 $\pm$ 0.1cm). With regard to dry extraction, it was found that methanol extract had the highest inhibiting activity with a zone of inhibition (1.7  $\pm$  0.2 cm), followed by dichloromethane (1.5 $\pm$ 0.0cm) and the least activity from hot water extract (1.1  $\pm$  0.3 cm). In Tathleeth B (Fig. 2B), cyclohexanone of fresh extract had the highest activity with a zone of inhibition (1.2  $\pm$  0.1 cm), followed by dichloromethane (1.0  $\pm$  0.0 cm), and less activity from chloroform extract (0.8  $\pm$  0.0cm). Dry stem extraction showed that diethyl ether had the highest inhibiting activity (2.1  $\pm$  0.1cm), followed by dichloromethane and chloroform extracts (1.7  $\pm$  0.0 cm) and (1.6  $\pm$  0.0cm) respectively whereas the least activity from methanol extract (0.9  $\pm$  0.1cm). Fig. 2C, revealed that fresh extract from Tabala C had the highest inhibiting activity from hot water extract (1.5  $\pm$  0.1cm), followed by methanol (1.4  $\pm$  0.1cm), chloroform (1.3  $\pm$  0.1 cm) and the least activity from cyclohexanone extract (0.8  $\pm$  0.1 cm). Dry extraction showed that the dichloromethane extract had the highest inhibiting activity (1.7  $\pm$  0.0 cm), followed by diethyl ether (1.6  $\pm$  0.1cm), whereas the least activity from hot water extract (0.7  $\pm$  0.5 cm). Fig. 2D

shows that Tabala D had the highest activity from hot water of fresh extract ( $1.8 \pm 0.4$ cm), followed by methanol ( $1.6 \pm 0.0$  cm) and the lowest activity from dichloromethane extract ( $0.6 \pm 0.1$ cm). Chloroform extract had the highest activity ( $2.0 \pm 0.0$ cm), followed by dichloromethane ( $1.7 \pm 0.0$  cm) and diethyl Ether ( $1.5 \pm 0.0$  cm) and the least activity from hot water extract ( $0.7 \pm 0.1$ cm).

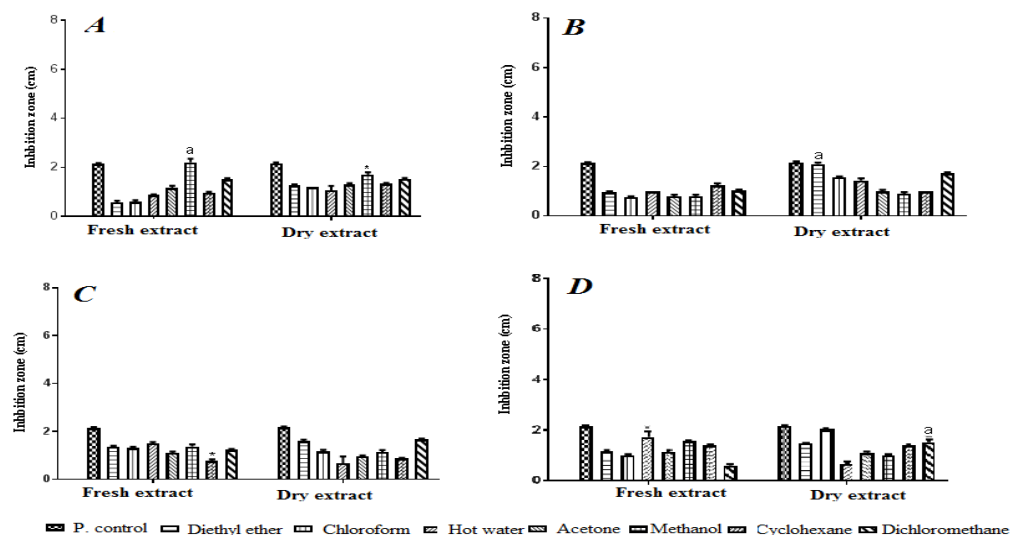


Fig. 2. Susceptibility of *C. albicans* to various solvent extracts of fresh and dry stem of *Commiphora gileadensis* grown in various locations. A, Tathleeth A; B, Tathleeth B; C, Tabala C; D, Tabala D. Mean value  $\pm$  SD, n= 3 (clearance zones of inhibition in cm). No symbol above column ( $P < 0.001$ ) and symbol (\*) ( $P < 0.05$ ) and (a) not significant compared with positive control.

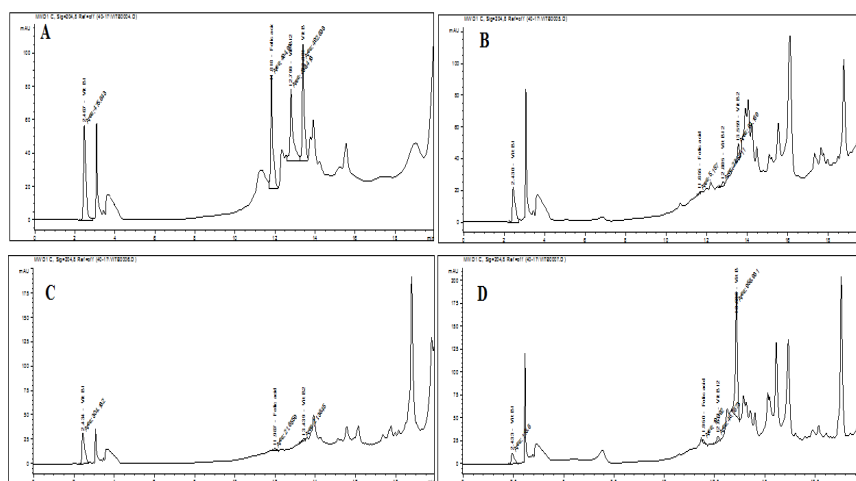
GC-MS analysis of stem ethanol extracts of *C. gileadensis* growing at various locations in south western part of Saudi Arabia showed various chemicals (Table 1). Phytochemicals are characterized and identified with their retention time, peak area percent, and molecular weight. According to the analysis, there were three chemical components found in both Thathleeth A and B, four in Tabala C, and one in Tabala D. Peak area revealed that 5-Methyl-2(5H)-furanone and palmitic acid are the major compounds in Tabala C, while ethylamine and 1-Ethyl-1-methylcyclopentane are dominant compound in Thathleeth A. In Thathleeth B the most dominant are 4-Ethylheptane and Furan while Tabala D contains amitrole. Fig. 3 and 4 showed the results of vitamins B<sub>1</sub>, B<sub>12</sub>, B<sub>2</sub> and folic acid content in *C. gileadensis* stems using high performance liquid chromatography (HPLC). It was noted that Tabala D had the highest amount from B<sub>1</sub> (11.64 ug/ml) and folic acid (1.01 ug/ml), followed by Thathleeth B (6.46 ug/ml). B<sub>12</sub> is rich in Tabala C region (1.96 ug/ml) followed by Thathleeth A (1.72 ug/ml). B<sub>2</sub> is rich in Tabala C (2.62 ug/ml) followed by Thathleeth A (1.72 ug/ml).

Anti-candidal activity of *C. gileadensis* stem extract was evaluated by measuring the inhibition zone of various solvent from various locations in southwest part of Saudi Arabia. This research proofed that plant location, types of plant extract either fresh or dry and solvent applied affect greatly the anti-candidal inhibition activities. The highest activity from fresh stem against *C. albicans* found in Tathleeth A from methanol extract, in Tathleeth B from cyclohexanone extract and from hot water extracts in Tabala C and D. The dry extract also showed variation among the activities as in Tathleeth A from methanol extract, in Tathleeth B from diethyl ether extract, in

Tabala C from dichloromethane extract and in Tabala D from chloroform extract. This variation in inhibition activity is likely the result of active chemical principles that can be extracted in various ways depending solvent capabilities or/and the environmental conditions that may have an impact on the synthesis of particular anti-candidal chemical compounds in specific location. Previous research showed that the antimicrobial effects gained from *Euryops arabicus* leaf extracts against distinct microbial strains varied in inhibition activities due to whether dry or fresh extract applied (Moustafa *et al.* 2018). Herein, there is fluctuation in chemical profiling of *C. gileadensis* as the production of specific chemicals affected greatly by plant locations. Moustafa *et al.* (2016) showed that plants of the same species might differ significantly in their phytochemical composition depending on the specific plant location.

**Table 1. GC-MS analysis of ethanol stem extract of *Commiphora gileadensis* from various regions.**

Region	Chemicals	Retenion time	% area	Molecular weight.	Chemical formula
Thathleeth A	Ethylamine	10.75	45.52	45.08	C <sub>2</sub> H <sub>5</sub> NH <sub>2</sub>
	alpha-Cubebene	11.107	16.89	204.357	C <sub>15</sub> H <sub>24</sub>
	1-Ethyl-1-methylcyclopentane	18.505	37.60	112.213	C <sub>8</sub> H <sub>16</sub>
Thathleeth B	FURAN	18.502	31.39	68.075	C <sub>4</sub> H <sub>4</sub> O
	4-Ethylheptane	22.204	42.95	128.255	C <sub>9</sub> H <sub>20</sub>
	Ethylamine	27.996	25.65	45.08	C <sub>2</sub> H <sub>5</sub> NH <sub>2</sub>
Tabala C	4E)-3-Methyl-4-hexen-2-one	16.849	5.051	112.170	C <sub>7</sub> H <sub>12</sub> O
	Cyclopropanecarboxylic acid	18.507	25.354	86.09	C <sub>3</sub> H <sub>5</sub> CO <sub>2</sub> H
	Palmitic acid	22.22	40.68	256.43	C <sub>16</sub> H <sub>32</sub> O <sub>2</sub>
	5-Methyl-2(5H)-furanone	28.015	28.90	98.10	C <sub>5</sub> H <sub>6</sub> O <sub>2</sub>
Tabala D	Amitrole	22.203	100	84.080	C <sub>2</sub> H <sub>4</sub> N <sub>4</sub>



**Fig. 3. Typical HPLC chromatogram of water soluble vitamins (B<sub>1</sub>, B<sub>12</sub>, B<sub>2</sub> and folic acid) of *Commiphora gileadensis* stem grown in Thathleeth A (A); Thathleeth B (B), Tabala C (C); Tabala D (D).**

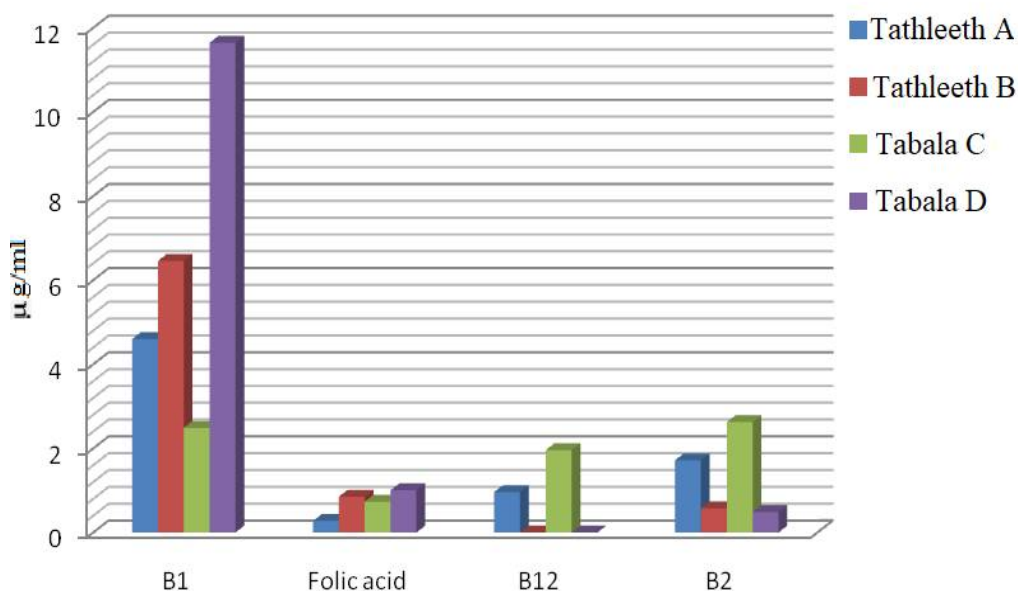


Fig. 4. HPLC analysis of water-soluble vitamins (B<sub>1</sub>, Folic acid, B<sub>12</sub> and B<sub>2</sub>) of *Commiphora gileadensis* grown in various regions in south western part of Saudi Arabia.

Results gained from HPLC showed there are changes in the quantity of each vitamin where *C. gileadensis* as well as variations in soluble vitamins including B<sub>1</sub>, B<sub>12</sub>, B<sub>2</sub> and folic acid. These variations may be related to the genotypes of the plants or physical growing factors, such as temperature, rain and humidity or to edaphic factors. Chemicals found in the extract of *C. gileadensis* showed biological significance, such as palmitic acid, which is well recognized to have significant antibacterial and antifungal activities (Chandrasekaran *et al.* 2011). Also, methanol and hot water are more potent to extract chemicals having antimicrobial activities (Bacon *et al.* 2017, Gebreyohannes *et al.* 2019). Methanol extract of *Ginkgo biloba* showed the highest antibacterial activity against *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Escherichia coli*, *Erwinia chrysanthemi* and *Xanthomonas phaseoli* than other extracts (Sati and Joshi 2011). Other study also showed that hot water extract was more efficient against *E. coli*, *Enterobacter*, *Klebsiella*, *Shigella* and *Salmonella* sp. (Behlül *et al.* 2014, Dhama *et al.* 2014). However, the diversity of inhibition zones according to the extract solvents may be reverted to the polar and non-polar solvent used. Moustafa and Alrumman (2015) and Wendakoon *et al.* (2012) reported that both the solvent types and the sample extraction conditions greatly affect the degree of antimicrobial activity. Researchers had explained this finding to the high volatility of some specific organic solvents, which tends to precipitate more bioactive chemicals from the plant sample than others (Oniszczuk and Podgórski 2015, Wrona *et al.* 2019).

There were variation among fresh and dry extracts, in agreement with previous findings indicated that fresh extract gained from *Rosa abyssinica* R. Br. ex Lindl. (Rosaceae) plant had the maximum antibacterial properties against *Shigella flexneri* and *Klebsiella pneumonia* (Moustafa and Alrumman 2015). As a starting point for developing and validating a biomarker at the species level in the study area, the differences in chemical and biological activity within various populations occurring in various sites might be utilized.

In conclusion, it is necessary to evaluate an organism's biological activities in a particular area. This study also holds an importance in using *C. gileadensis* stem extract as an alternative source for treating diseases generating from *C. albicans* and provides support to the plant's traditional and alternative use.

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