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REVIEW

IMPACT OF COVID-19 PANDEMIC ON DIETARY HABITS AMONG ADOLESCENTS AROUND THE WORLD: A SYSTEMATIC REVIEW"

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ABSTRACT

The current research aimed to investigate the influence of Covid-19 lockdown on eating behavior changes among adolescents worldwide.

A comprehensive search strategy was implemented, and electronic literature searches were carried out using the web of sciences, PubMed, and Scopus to classify published articles between the appearance of the COVID-19 Pandemic at the end of the year 2019 and November 4, 2021, in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) standards.

The results showed that there were significant changes in eating behaviors among adolescents during the COVID-19 Pandemic stages, and this change included significant decreases in the intake of dairy products, meat, fish, eggs, vegetables, and fruits as well as clear increases in the consumption of snacks, hamburgers, candies, soft drinks. There were significant differences in eating behaviors due to the difference in adolescents' sex.

The current review shows significant changes in eating behaviors during the Covid-19 Pandemic. The review also showed significant gender differences in food patterns of an adolescent during the COVID-19 Pandemic.

KEYWORDS:

Covid-19 Pandemic, dietary habits, adolescents

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is a viral disease caused by severe acute respiratory syndrome coronavirus 2 (novel SARS CoV-2 virus), which is a single-stranded enveloped positive-sense RNA virus1, that began in Wuhan, China, and has to propagate to all major cities and countries worldwide [1,2]. As of May 28, 2020 (11.52 GMT), COVID-19 had infected more than 5.8 million people in 213 countries and territories worldwide, as well as two international conveyances, with an estimated death toll of 0.357 million [3]. Throughout the COVID-19

Pandemic. According to the Centers for Disease Control and Prevention (CDC), nearly one-third of U.S. adults will suffer from anxiety or depression by June 2020 [4].

Restrictions and changes in lifestyle during lockdown have been linked to lower psychological well-being. Reduced social contact, feelings of isolation, and fear of contracting or spreading dangerous viruses have all been linked to poor mental health in the past [5,6]. It has been demonstrated that time spent in quarantine during previous disease outbreaks has a negative psychological impact [7]. Poor sleep quality has been linked to the COVID-19 Pandemic in China [8] and Italy [9], with more than half of Italian respondents reporting poor sleep quality. In Spain, regrettably, the quality of respondents' sleep appeared to improve as the lockdown progressed [10]. Access to fresh food has been restricted since the start of the COVID-19 Pandemic, and people are spending more time indoors and limiting their physical activity [11]. Stress and anxiety have been shown to increase the consumption of alcohol and sugary foods, and energy imbalance is also possible because energy expenditure during lockdown is reduced [12]. Positive lifestyle habits, such as more time for cooking and less fast-food consumption, may have emerged due to the Pandemic [11]. Regrettably, boredom and stress are linked to increased food intake, particularly sugary comfort foods. Food cravings are more common in women than in men. Carbohydrate craving raises serotonin levels, which improves mood and can be used to combat stress. This unhealthy dietary routine may increase the risk of chronic medical conditions such as obesity, diabetes, and lung disease, all of which can exacerbate COVID-19 complications in a community [12]. Concerning dependent people such as the elderly, people with extreme obesity, and people with other physical disabilities, scientific evidence has linked precautionary measures of physical contact restriction to changes in eating behavior. These changes have been linked to a significant decline in health nutrition [13]. Therefore, the current review sought to investigate the impact of Covid-19 lockdown on eating behavior changes in adolescents worldwide.

MATERIALS AND METHODS

Search Strategy. A systematic search strategy was developed, and electronic literature searches were conducted using the web of sciences (www.webofknowledge.com), PubMed (www.Pubmed.org), and Scopus (www.scopus.com) to identify papers published between emerge of Covid-19 at the end of the year 2019 to November 4, 2021, following the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guidelines. Searches were conducted using the following combinations of keywords: dietary habits or diet and Covid-19 or Coronavirus and adolescents OR teenager.

Study Selection. After removing duplicate articles, two independent reviewers screened the titles and abstracts to select only those studies that met the inclusion criteria. The full articles were obtained for the screen of eligibility.

Eligibility Criteria. Eligibility criteria were articles reporting dietary information with different measuring methods for healthy adolescents during corona, data from any study design, and published in English. Articles were excluded if reported not enough data on diet or diet were not measured during corona various, articles were populations not adolescents

Data Extraction. Data extracted from articles were compiled into Microsoft Office Excel 2016 data file. Information extracted included: author, country, population, age, sample size, study design, dietary intake measure, and outcomes. An independent third reviewer checked and approved the accuracy of extracted data to minimize any imputation errors.

Quality Assessment. The quality of final articles was assessed using The Newcastle-Ottawa Scale tools for observational studies based on (overall quality, study population, methods, results, and risk bias), with each area containing questions relevant to the quality of the article. Scores were given for each area as +1 for addressing the issue appropriately, -1 for not addressing the issue, and 0 if it was unclear. Studies were defined as being of satisfactory quality if they scored ≥ 7 and were therefore deemed suitable for inclusion. Two reviewers assessed the quality assessments of included studies.

RESULTS

Characteristics of Included Studies. Based on our research strategy, 298 records were identified from the initial searches. After removing duplicates, 229 records were screened by title and abstract, with 163 of these papers excluded as they did not fit the inclusion criteria, leaving 66 full-text articles obtained and assessed for eligibility. Of these 66 articles, 42 were excluded getting only 24 articles. The following diagram of the study selection process is outlined in Figure 1. The studies included in this review were from different countries: Italy (n=7), Spain (n=4), Poland (n=3), Korea (n=3), and one study each from the following countries China, Greek, Australia, Croatia, Japan, Brazil and one study from different countries (Spain, Italy, Brazil, Colombia, and Chile).

Dietary Intake Measures. The studies included in this review included different measures and assessment of dietary intake. The study by Dragun et al. [13] was a questionnaire on 2020 to estimate the changes in dietary behaviors during the Covid-19 lockdown compared to the period before lockdown. Horikawa et al. [15] study measured intakes of selected food groups before, during, and after a COVID-19 state of emergency. The third study included in this review by Teixeira et al. [16] was a standardized questionnaire (PeNSE) to measure the frequency of consumption. Respondents reported food groups or preparations consumed during social isolation in the last 7 days. Munasinghe et al. [17] study, self-reported dietary behavior at baseline was measured using questions validated for adolescents by the NSW Centre for Public Health Nutrition, and self-reported dietary behaviors relating to the previous 24-hour period were also collected each week for the 22-week follow-up period via a momentary ecological assessment (EMA).

Morres et al. [18] studied the short diet behavior Questionnaire for Lockdowns (SDBQ-L). Following a short description of what consists of healthy eating, participants were asked to indicate whether (a) they were following a healthy diet, (b) they were eating more than usual, (c) they were eating on a consistent schedule, and (d) they were eating out of control. A study by Lee et al. [19] and Yoo et al. [20] included diet habits. In addition, a study by Kim et al. [21] was surveyed in 2019 and 2020 to assess dietary habits of breakfast, fresh fruit, and soda beverage frequencies. A study by Pietrobelli et al. [22] was a question that included eating habits focusing on servings of red meat, pasta, snacks, fruits, and vegetables.

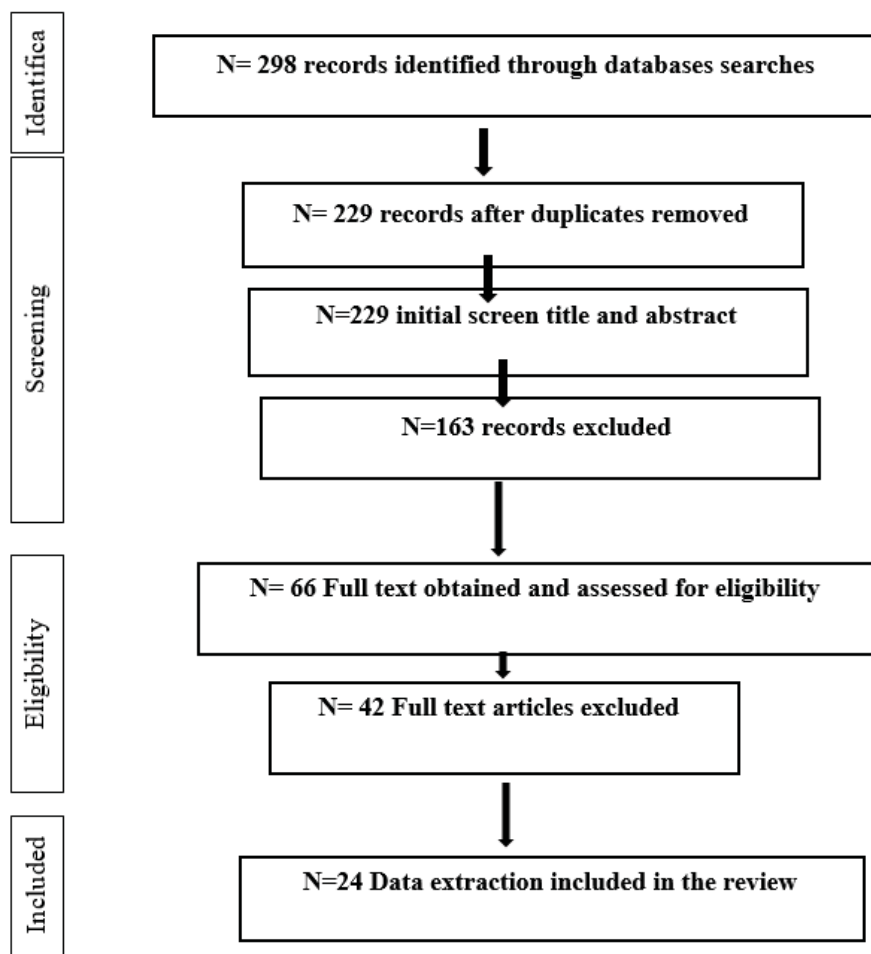


FIGURE 1
PRISMA Flow Diagram of the systematic literature search and article selection.

Mastorci et al. [23] evaluated dietary habits using the Mediterranean Diet Quality Index for adolescents (KIDMED). The KIDMED index was based on principles sustaining or undermining the Mediterranean diet approach [for example, “Every day I eat fruit or freshly squeezed fruit juice,” “Regularly once a day would consume fresh and cooked vegetables,” “I eat pasta and rice almost every day (5 or more per week)”]. The index ranged from 0 to 12 and consisted of a self-administered 16-question test. The validity of the KIDMED index is demonstrated by the evidence that a higher score is associated with the expected patterns of food and nutrient intake, representative of a good quality diet.

In a study by Nicodemo et al. [24], participants had to fill in the dietary habits questionnaire with a parent, such as eating breakfast, the presence of two snacks in the day, healthy meals at lunch and dinner, drinking water, doing home workouts.

Moreover, in the study by Galluccio et al. [25], the study's online questionnaire included the KIDMED test, used to assess adherence to the Mediterranean diet (M.D.). The MD adherence score, from 0 to 12, was based on a 16-point test. Questions denoting a positive aspect concerning the M.D. were

assigned a value of +1 (consumption of fruits, vegetables, fish, legumes, whole cereals or grain, nuts, oil, dairy products, and yogurt) and those with a negative connotation -1 (skipping breakfast, consumption of baked goods, sweets, and consumption of delivery junk foods). The level of adherence to M.D. was indicated as follows: high adherence to M.D. (≥ 8 points), medium adherence to MD (4–7 points), and low adherence to M.D. (≤ 3 points).

Pujia et al. [26] prepared a questionnaire that included eating habits (the frequency of milk, dairy, vegetable, legume, fruits, meat, fish, egg, pasta, rice, bread, pizza, sweet, oil, margarine, butter, and soft beverage consumption before and during the lockdown). We used the same method but a modified version of the questionnaire of the Italian council for research in agriculture and agrarian economics (CREA), which was previously used in a survey.

In a study by Salzano et al. [27], the war part of the questionnaire focused on participants' eating habits. In addition, Cipolla et al. [28] study were telephone interviews lasted about 20 minutes and included a questionnaire on eating habits (number of

meals/days, quantity of food taken, daily consumption of certain foods-for-example junk food consumption, pizza, sweets, red meat, fruits, and vegetables).

The study of Jia et al. [29] used foods surveyed included 12 major groups commonly consumed by the Chinese: rice, wheat products, other staple foods, meat, poultry, fish, eggs, dairy products, fresh vegetables, preserved vegetables, fresh fruit, and soybean products; this food frequency questionnaire has been validated in the China Kadoorie Biobank Study [30]. Participants were asked to self-recall their frequency of food consumption before and under COVID-19 lockdown by choosing one of the four categories of frequency (“daily”, “4–6 days per week”, “1–3 days per week”, “none”). They were also asked to report the frequency of consuming beverages most frequently consumed in the two periods, i.e., sugar-sweetened beverages (SSBs), coffee and caffeinated drinks, tea, and other beverages.

A study by Ruiz-Roso et al. [31] was an online questionnaire comprising more than 30 questions about dietary habits during COVID-19 confinement and the previous period. The adolescent recorded the number of days on which they consumed the following foods or food groups during the week before confinement (BEFORE) and one week during confinement (COVID19): legumes; vegetables; fruit; sweet food; fried food (including packaged potatoes); processed meat (burger, sausage, mortadella, salami, ham, chicken nuggets, or sausages); sugar-sweetened beverages (SSB), and fast food.

For Ventura et al. [32] study, where different sections of the questionnaire aimed to examine: (i) adherence to the Mediterranean diet (AMD) after the implementation of lockdown. Adherence to the Mediterranean diet (AMD) was assessed through the Mediterranean diet quality index for children and adolescents (KIDMED score). Details on how the score was calculated are described elsewhere [8]. The KIDMED scores ranged from -4 to 12 points, where higher values denote higher AMD. Score values can then be categorized into: (i) high or optimum AMD (KIDMED 8 to 12); (ii) medium AMD (KIDMED 4 to 7); and (iii) low AMD (KIDMED equal or less than 3 points). In our study, we considered both the KIDMED score and categories of AMD for describing dietary habits after the implementation of lockdown.

The study by Aguilar-Martínez et al. [33] was self-administered by email or WhatsApp to high school students. Based on the score obtained (range 0–100), the quality of the participants’ diets was divided into three categories: unhealthy diet (<50 points); a diet that needs changes (50–80 points); healthy diet (>80 points). To determine whether changes in diet and eating behaviors had occurred during the COVID-19 pandemic confinement, for each variable, we asked whether the participant’s

frequency of consumption or behavior had decreased, remained unchanged, or increased compared to the pre-pandemic period.

In the study by Martín-Rodríguez et al. [34], nutritional habits were analyzed using an adapted previously used questionnaire. The first 2 questions were related to eating habits. The rest of the questions were related to the consumption frequency of different food groups, including fish, vegetables, legumes, meat, fast food, or soft drinks. Each answer was a score ranging from 1 to 6, where 1 = “I do not consume”, 2 = “less than three per week”, 3 = “three or more per week”, 4 = “seven or more per week”, 5 = “ten or more per week”, 6 = “more than thirteen.

In Villodres et al. [35] study, the KIDMED test was used to evaluate M.D. adherence. The latest updated version adapted into Spanish was used. It consists of 16 items designed to measure levels of M.D. adherence in children and adolescents. Items must be answered affirmatively (yes) or negatively (no). Four of the items are negatively framed, with positive responses scoring -1. In contrast, positive responses to positively framed items are scored +1. Negative responses have a score of 0. Thus, the total index ranges from -4 to 12. From this, M.D. adherence is categorized along the following continuum: Poor quality (≤ 3); Needs improvement (4–7); Optimal quality (≥ 8).

The study by Skolmowska et al. [36] used the scores of the Adolescents’ Food Habits Checklist (AFHC) before and during the pandemic in the Polish Adolescents’ COVID-19 Experience (PLACE-19) Study population. The participants were required to complete an AFHC consisting of 23 items about food purchase, preparation, and consumption habits.

In Guzek et al. [37] study, the food preferences were assessed using the Food Preference Questionnaire (FPQ). The FPQ includes a list of 62 various food items to be defined on how much, on average, the respondent like the specific item with the possible answers as follows: (1) dislike a lot, (2) dislike a little, (3) neither like nor dislike, (4) like a little, (5) like a lot (for any food item they have ever tried, independently from the actual consumption), as well as (6) not applicable (for any food item they don’t know or don’t remember ever having tried). The FPQ allows for assessing the preferences of vegetables (the questionnaire includes 18 food items in this food category), fruit (7 items), meat/fish (12 items), dairy (10 items), snacks (9 items), and starches (6 items), which may be obtained by summing the single food preference item scores within each food category and dividing this sum by the number of items.

In addition, the appetitive traits were assessed while using Adult Eating Behavior Questionnaire (AEBQ) [38]. The AEBQ is a validated self-report tool to be applied in the case of adolescents and adults, which was developed to assess food approach

and food avoidance. The AEBQ includes a list of 35 items to be defined using a 5-point Likert scale (from 'strongly disagree' to 'strongly agree'). The AEBQ allows for assessment following food approach subscales: hunger (5 items), food responsiveness (4 items), emotional over-eating (5 items), enjoyment of food (3 items), and following food avoidance subscales: satiety responsiveness (4 items), emotional under-eating (5 items), food fussiness (5 items), slowness in eating (4 items). The scores for each scale are obtained by attributing points to each item—depending on a question, either from 1 to 5 points or from 5 to 1 point, and by calculating the mean score for each subscale.

For the other study in Poland as well, Głowska et al. [39] used the FPQ allowing the assessment of preferences for the following categories of food products: vegetables, fruit, meat/fish, dairy, snacks, and starches, based on the information provided for specific food items within the listed categories (vegetables—18 items, fruit—7 items, meat/fish—12 items, dairy—10 items, snacks—9 items, starches—6 items). A total of 62 food items are included, while each of the respondents is asked how much they enjoy it on average. For each food item, the respondent chooses one of the following answers: (1) dislike a lot, (2) dislike a little, (3) neither like nor dislike, (4) like a little, (5) like a lot, and (6) not applicable (the last answer should be indicated for each food item that the respondent does not know, or does not remember ever having tried, while for any other they should choose the answer defining specific preference). Based on the answers provided for specific items (close-ended questions), the preferences for food categories are calculated by summing the single item preference scores within each food category and dividing this sum by the number of items. The FCQ allows the assessment of the choice determinants in the following categories: health, mood, convenience, sensory appeal, natural content, price, weight control, familiarity, and ethical concern based on the answers about how important respondents consider 36 items while choosing the food they eat during a typical day. The included items describe specific features of food attributed to the listed food choice determinants (health—6 items, mood—6 items, convenience—5 items, sensory appeal—4 items, natural content—3 items, price—3 items, weight control—3 items, familiarity—3 items, and ethical concern—3 items). At the same time, for each of them, the respondent is asked about how important it is for them to consume, on a typical day, a food item that presents specific features. For each item, the respondent chooses one of the following answers: (1) not at all important, (2) a little important, (3) moderately important, and (4) very important. Each answer is attributed to a score from 1 (not at all important) to 4 (very important). Based on the answers provided for specific items (close-ended questions), food choice determinants are calculated

by summing the single item scores within each determinant and dividing this sum by the number of items.

DISCUSSION

The impact of the Covid-19 Pandemic on diet and dietary habits was assessed in the studies included in this review. In the study by Dragan et al. [14], during the COVID-19 lockdown, 324 Croatian adolescents modified their dietary patterns, with a reduction in fruits and vegetables of approximately 5.2 and 2.8, respectively, and an increase of 34.6 and 18.5, respectively, with the remainder being unchanged. Meat and processed meat were lowered by 7.7 percent, rose by 11.4 percent, and remained unchanged by 80.9 percent. Sweets and snacks were also amended, with 29.9 being decreased, 26.5 being raised, and 43.5 remaining unchanged. This explained the impact of Covid-19 on this group's eating habits.

Prevalence of consuming specific food categories at least twice a day based on the family income level of Japanese Schoolchildren aged 10–14 before, during, and after the COVID-19 state of emergency was evaluated. The quality of meals for kids was determined by household income level. Schoolchildren in all four family income groups consumed less milk and dairy products, meat, fish, eggs, vegetables, and fruits under the state of emergency than previously. Intake was also more prevalent after the COVID-19 state of emergency than during the state of emergency. In all four household income levels, the prevalence of consumption was identical before and during the state of emergency. The percentage of respondents who consumed a well-balanced diet at least twice a day was defined as "Well-balanced dietary intake" (defined as the intake of both meat, fish, or eggs and vegetables) was significantly lower in all income quartiles during the state of emergency compared to those before the state of emergency [15].

In the study conducted in Brazil by Teixeira et al. [16], the results showed that children consumed more often than adolescents ($p < 0.001$). Children ate their meals more frequently ($p < 0.001$) than adolescents, who frequently exchanged substantial meals for snacks. Compared to adolescents ($p = 0.001$), children in isolated homes consumed more raw salad ($p = 0.039$), vegetables ($p = 0.001$), beans ($p = 0.025$), and fresh fruits and fruit juices ($p = 0.002$). Adolescents drank more hamburgers, candies, soft drinks, and sugar-sweetened beverages than children ($p < 0.001$).

A study conducted in Australia by Munasinghe et al. [17] indicated significant decreases in fast food consumption following the installation of physical distancing. Still, there were no significant changes in fruit and vegetable intake.

The study was conducted on 950 Greek secondary and high school students, the number of male students was 432, and the number of female students was 518. The results showed that the mean eating behavior assessment for boys and girls was 4.38 1.18 and 4.53 1.24, respectively. There were no detectable effects on healthy eating habits [18].

The study was conducted on 333 Korean middle school adolescents, the number of male students was 153, and the number of female students was 180. Diet had a mean value of 4.42 and a performance score of 3.89. There was a substantial positive correlation between the importance of all subdimensions of health perception, such as diet, and the performance of each dimension of health perception. There were significant differences in priority and competence for all items in the mental health, illness, physical activity, sleep, and food dimensions. The reported importance ratings were greater than the reported performance scores for all items [19]

The mean score of diet and lifestyle behavior management was 4.42 and 3.89 in importance and performance, respectively, for respondents in assignment-based online P.E. classes, and 4.50 and 3.87 in importance and performance, respectively, for respondents in interactive P.E. classes. There were significant differences in the importance and performance of all health awareness factors (diet and lifestyle habit management) among students who took assignment-based and interactive online P.E. classes [20].

In Korea, there was study included 105,600 participants (53,461 in 2019 Male 27,776 Female 25,685; 52,139 in 2020 Male 27,033 Female 25,106). The 2020 group had reduced rates of consuming fruit, fast food, drinking soda, and drinking sweet beverages in the fully adjusted model than the 2019 group (all $p < 0.001$). On the other hand, breakfast frequency was lower in the 2019 group than in the 2020 group ($p < 0.001$). In the 2020 group, the frequency of eating fruit, drinking soda, drinking sweet beverages, and eating fast food was lower than in the 2019 group (all $p < 0.001$) [21]

Italy 2020 The number of meals taken per day significantly rose by 1.15 ± 1.56 ($p < 0.001$). Vegetable intake remained unchanged, whereas fruit intake increased (marginal significance, $p = 0.055$). During the lockdown, consumption of potato chips, red meat, and sugary drinks rose significantly ($p = 0.005$ – 0.001). There was an inverse relationship between changes in sports involvement and changes in meal frequency ($r = 0.35$, $p = 0.027$) and screen time ($r = 0.27$, borderline significant at $p = 0.084$). The only gender difference in lifestyle question responses was for a change in meals/day between males and females. The number of meals consumed per day rose significantly higher in men (1.64 ± 1.65) than in women (0.581 ± 1.26 ; $p = 0.028$) [22]

In Italy, a study included 1,289 (male, 622 female, 667) by Mastorci et al. [23]. Regarding lifestyle, adolescents' adherence to the Dietary pattern during COVID-19 quarantine (C.Q.) than during standard conditions (mean change = 0.4, CI: 0.3–0.5, $t = 6.4$, $p < 0.001$). (S.C.). Compliance with the Dietary Pattern has been increased in the C.Q. monitoring (mean change = 0.5, CI: 0.3–0.6, $t = 5.3$, $p < 0.001$). During the lockdown, lifestyle habits were characterized by an increase in adherence to the Mediterranean diet (mean change = 0.4, CI: 0.2–0.5, $t = 3.8$, $p = 0.001$) and a low level of physical (mean change = 0.1, CI: 0.2 to 0.1, $t = 2.7$, $p = 0.008$).

Italy 88 32 male patients (36.4%) and 56 female patients (63.6%) with obesity (body mass index (BMI) 27.9 kg/m² (SD 4.4). Breakfast meals have been eaten by 85.2 percent of the population. Whereas there was an equitable distribution of eating a midmorning snack (29.5 percent "yes," 30.7 percent "no," and 39.8 percent "sometimes"), an afternoon snack was eaten up by 81.8 percent of the population. The fruit was the most popular afternoon snack (64.3%), followed by biscuits, pizza, and cold cuts. Vegetable consumption was significantly greater at dinner (69.3 percent) than at lunch meal (48.9 percent) (Table 1), suggesting that 46.6 percent of our population consumes vegetables at both meals [24].

In terms of eating habits during the COVID-19 lockdown of Italian adolescents 91 (42 females and 49 males), the majority of people declared no variations (total sample: 63%, females: 59%, males: 65%), while the proportions of total adolescents who consumed 4 and 5 meals/day were 39% and 26%, respectively. The KIDMED questionnaire, which assessed adherence to M.D. recommendations, discovered an average adherence score in total adolescents, without gender differences, with approximately 50 % of the population proclaimed medium adherence to the M.D. (medium adherence: total sample: 49 percent, female 48 percent, male 51 percent). For most dietary recommendations, there were significant differences in food rate changes between active and sedentary adolescents. Indeed, active adolescents consumed more fruit per day, a second fruit per day, more vegetables per day, nuts twice a week, and low-fat dairy products for breakfast than sedentary adolescents. Indeed, active adolescents consumed more fruit per day, a second fruit per day, more vegetables per day, nuts twice a week, and low-fat dairy products for breakfast than sedentary adolescents. The percentages of subjects who ate breakfast and did not consume sweets or candy daily were significantly higher in the same group of adolescents than in the other groups [25].

Food patterns were described during the first COVID-19 lockdown of Italian adolescents aged 10-14 years. During the lockdown, a considerable number of parents reported that their children's eating habits had altered. Children and teens who gained

body weight had a more sedentary lifestyle and greater variation in height (Table 2), and the adolescents who gained body weight reported increased consumption of milk/cheese/yogurt; processed meat; bread/pizza/baked goods; and soft drinks. Pearson's correlation revealed that body weight gain in adolescents was related to age ($r = 0.13$; $p = 0.05$), body height at baseline ($r = 0.26$; $p = 0.02$), physical activity change ($r = 0.25$; $p = 0.001$), milk/cheese/yogurt consumption ($r = 0.15$; $p = 0.03$), meat/fish/egg consumption ($r = 0.12$; $p = 0.08$), and processed meat consumption ($r = 0.18$; $p = 0.01$). Moreover, body weight gain was associated with bread/pizza/bakery product consumption ($r = 0.18$; $p = 0.01$), ice cream/dessert consumption ($r = 0.13$; $p = 0.06$), packaged sweet snack consumption ($r = 0.23$; $p = 0.04$), candy consumption ($r = 0.12$; $p = 0.08$), chocolate consumption ($r = 0.13$; $p = 0.06$), and sweet beverage consumption ($r = 0.16$; $p = 0.06$). Weight gain did not correlate with basal BMI ($p = 0.31$). According to Pearson's correlation, BMI gain in adolescents was only correlated with processed meat ($p = 0.08$) and candy consumption ($p = 0.03$) [26].

In Italy, Salzano et al. [27] assayed the changes in eating habits among 1860 Italian adolescents aged from 12 to 18 years. The results showed that 47.5 percent of adolescents stated that their eating patterns had not been modified. Furthermore, 25.6 percent of those surveyed reported a more balanced diet, while 26.8 percent mentioned their eating patterns had deteriorated.

Cipolla et al. [28] evaluated the meal changes of Italian adolescents. 33 (51.6%) patients were overweight (BMI between 85th and 95th percentile), while 31 (48.4%) were obese (BMI > 95th percentile). The results showed that more than 50 % of participants (67.2 percent) increased their consumption of bread, pasta, and pizza during the lockdown, particularly patients with $\text{BMI} > 0$. Indeed, there was a significant difference between the groups regarding carbohydrate intake versus fiber, vegetables, fruit, or proteins ($p = 0.002$). During the Pandemic, about a third of patients (31.2 percent) drank sugary drinks, with no statistically significant differences between the two groups. There were no statistically significant differences in the number of daily meals; most patients consumed 4 or 5 meals per day. A relatively poor diet was discovered in 61.3 percent of patients with BMI gain versus 12.1 percent of those with BMI decrease ($p = 0.000$). Additionally, 42% of children and adolescents reported that they had consumed meals other than normal during the pandemic and that they had often prepared dishes using recipes found online. Fifty-six percent said they never prepared meals independently because the parent was mainly at home, worked smartly, and had more time to dedicate to them.

In China, Peng, 2020 studied the changes in food intake frequency during the COVID-19 pandemic. The results showed that during the COVID-

19 lockdown, significant decreases in the frequency of intake of rice, meat, poultry, fresh vegetables, fresh fruit, soybean products, and dairy products were observed (all p -values 0.05). Significant increases in bakery product consumption, other staple foods, and canned vegetables were observed (all p -values 0.01); males consumed these foods more frequently than females. Males consumed more beverages than females. Regarding beverage type, most participants consumed SSBs in both periods, though the percentage had decreased slightly under lockdown [29].

The dietary habits during the COVID-19 pandemic were evaluated by Ruiz-Roso et al. [31] in Spain, Italy, Brazil, Colombia, and Chile. More than 30 questions about dietary habits during COVID-19 confinement and the previous period were addressed in an online questionnaire. The National School Health Survey–PeNSE; Pesquisa Nacional de Saude do Escolar [40], which was slightly modified, was used to assess adolescent dietary practices. A questionnaire was used to collect data, divided into modules: sociodemographic and family characteristics and dietary practices before and during confinement. During the week before confinement (BEFORE) and one week during confinement (COVID19), the adolescent recorded the number of days they consumed the following foods or food groups: legumes; vegetables; fruit; sweet food; fried food (including packaged potatoes); processed meat (burger, sausage, mortadella, salami, ham, chicken nuggets, or sausages); sugar-sweetened beverages (SSB), and fast food.

In Spain, Ventura et al. [32] evaluated (i) adherence to the Mediterranean diet (AMD) after the implementation of lockdown. The assessment depended on (the KIDMED score). they stated that Low (score < 4) 35 (3.8) medium (score 4–7) 413 (45.4) High (Score ≥ 8) 462 (2.1) KIDMED score, mean (SD) 7.4 ± 2.1 .

Aguilar-Martínez et al. [33] evaluated the changes in diet and eating behaviors during the COVID-19 pandemic confinement in Spain. Approximately 40% of participants reported an increase in fruit and vegetable intake. Contrary, intake of sweets and pastries, convenience foods, and soft drinks decreased by 39.3, 49.2, and 49.8 percent, respectively, across the entire sample. Regarding food-related behaviors, 21.5 percent of the participants reported an increase in the variety of foods. In contrast, 56.4 percent of adolescents reported consuming more snacks between meals, 39.9 percent reported less regularity in meal distribution, and more than half reported changes in meal frequency (28.4 percent increased the number of meals versus 22.4 percent who decreased them). Eating snacks between meals was the behavioral variable that increased the most during confinement in both girls and boys [54.5 percent (95 percent CI = 47.7–61.1 percent) and 61.1

percent (95 percent CI = 50.7–70.6 percent), respectively]. The most decreased behavioral variable was mealtime regularity [41.3 percent (95 percent CI = 34.9–48.1 percent) for girls and 36.7 percent (95 percent CI = 27.3–47.1 percent) for boys]. In terms of food consumption, the results showed the greatest increase in fruit consumption [42.7 percent (95 percent CI = 36.2–49.5 percent) in girls and 30.0 percent (95 percent CI = 21.4–40.3 percent) in boys]. Furthermore, the results also indicated that girls had the greatest decrease in convenience food consumption [50.2 percent (95 percent CI = 43.5–56.9 percent)]. Sweets consumption was the variable that decreased the most in boys [41.1 percent (95 percent CI = 31.4–51.6)]. Before confinement, the average HEI score of the participants was 68 points. Significant gender differences in HEI prevalence before COVID-19 confinement were observed. In girls, 12.2 percent had a healthy diet, 79.8 percent needed to make some changes, and 8% had an unhealthy diet; in boys, these proportions were 2.2, 93.3, and 4.4 percent, respectively (p-value 0.01). The other variables investigated revealed no statistically significant differences. The results are broken down into the Spanish Health Eating Index (HEI) textiles. There are no statistically significant differences in food consumption or eating behavior based on dietary quality before COVID-19 confinement.

During the COVID-19 pandemic Martín-Rodríguez et al. [34] evaluated the change in eating habits of Spanish adolescents. Females consumed more fast food and bakery products than males, who consumed more vegetables, rice, pasta, legumes, fish, milk, cheese, beer, and alcohol. A significant negative correlation was found between loneliness (UCLA) and alcohol consumption (r: 0.203, p: 0.024), experimental avoidance and psychological inflexibility, and dry mouth or lack of saliva values (r: 0.349, p: 0.000). In terms of dietary habits, a negative correlation was discovered between the number of meals per day and BMI (r: 0.240, p: 0.006). Furthermore, lower levels of fruit consumption were discovered when levels of alcohol consumption were higher (r: 0.251, p: 0.005). Females consumed more fast food and bakery products than males, who consumed more vegetables, rice, pasta, legumes, fish, milk, cheese, beer, and alcohol. A significant negative correlation was found between loneliness (UCLA) and alcohol consumption (r: 0.203, p: 0.024), experimental avoidance and psychological inflexibility, and dry mouth or lack of saliva values (r: 0.349, p: 0.000). In terms of dietary habits, a negative correlation was discovered between the number of meals per day and BMI (r: 0.240, p: 0.006). Furthermore, lower levels of fruit consumption were discovered when levels of alcohol consumption were higher (r: 0.251, p: 0.005). Furthermore, there was a significant positive correlation between alcohol consumption and bakery product consumption (r: 0.234, p: 0.009), fast food intake (r: 0.360, p: 0.000), mobile

usage (r: 0.215, p: 0.016), and smoking habits (r: 0.215, p: 0.016). (r: 0.227, p: 0.011). Moreover, correlation analysis revealed a significant positive correlation between female weight and alcohol consumption (r: 0.298, p: 0.021) and a significant negative correlation between meal frequency and alcohol (r: 0.507, p: 0.000). The data revealed a significant positive correlation between mobile usage and alcohol consumption (r: 0.362, p: 0.003), soft drink consumption (r: 0.472, p: 0.000), and rice consumption and weight (r: 0.258, p: 0.0038) in males. On the other hand, female students had higher mobile usage when beer consumption was indeed higher (r: 0.316, p: 0.014), and they also had higher BMI values when alcohol consumption was higher (r: 0.0257, p: 0.0047).

Villodres et al. [35] used the KIDMED test to evaluate M.D. adherence in Spanish people aged 15-20. The results showed no significant differences in sample sizes between boys and girls according to MD 6.17 2.48, and 5.92 2.93 P=0.139 There are no significant differences in MD 5.98 2.85 and 6.04 2.73 P=0.273 were observed among sample between total confinement and pre-confinement. Low socioeconomic status harmed M.D.'s adherence.

Current and previous habits (during the Pandemic) were assessed and scored separately. The total (p = 0.001), procurement (p = 0.001), and consumption scores (p = 0.014) clearly indicates that AFHC scores increased during the pandemic. AFHC scores increased during the Pandemic, a similar share of participants improved or worsened their food patterns, and a similar share changed their eating habits from healthy to unhealthy and unhealthy to healthy [36].

The categories with the highest attitudes in the studied group were fruit, snacks, and starches (median values greater than 4 points attributed to the 'like a little' category) and meat/fish (median value greater than 3 points attributed to 'neither like nor dislike' category). Dietary preferences for subgroups stratified based on clustering of preferences evaluated by the Food Preference Questionnaire (FPQ) within the population of low-preferring respondents (low preference for all food categories), participants generally prefer snacking foods (low preference for all food categories, except for fruit and snacks, as median values were higher than 4 points attributed to 'like a little' category), and high-preferring respondents (high prefer All appetitive traits differed between respondents from different clusters. The low-preferring participants (cluster 1) had the lowest values for all appetitive traits (p = 0.0008) as well as the total score (p = 0.0001), except food fussiness, which had the highest value (p = 0.0008). Snacking-preferring (cluster 2) and high-preferring (cluster 3) respondents shared similar traits of hunger and emotional overeating, while all other appetitive traits differed significantly (p =

0.0008). The vegetable preference score was associated with hunger, food responsiveness, emotional overeating, food enjoyment, emotional undereating, and eating slowness, while it was negatively associated with food fussiness [37].

Dietary preferences were evaluated using the FPQ for subgroups stratified based on the clustering of the assessed FCQ scores within the population of the PLACE-19 Study's second phase; When comparing clusters with different food choice determinants, preferences for all food categories differed. The "healthy eaters" (cluster 1) preferred vegetables the most; the "hedonists" (cluster 2) preferred meat/fish, dairy, and snacks; the "demanding consumers" (cluster 4) preferred all food categories, and the "indifferent consumers" (cluster 3) had a low preference for all food categories. Except for familiarity, the vegetable preference score was positively associated with all food choice determinants. All food choice determinants were positively associated with fruit preference scores. The meat/fish preference score was related to health, mood, convenience, sensory appeal, natural content, and price. The dairy preference score was positively associated with all food choice determinants except for weight control. Besides health and ethical concerns, snack preference score was positively associated with all food choice determinants. All food choice determinants were positively associated with the starches preference score [39].

CONCLUSIONS

The current review evaluated the changes in diet and consumption behaviors of food items among adolescents during the COVID-19 Pandemic stages. The current review shows significant changes in eating behaviors during the Covid-19 Pandemic. The review also showed significant gender differences in food patterns of an adolescent during the COVID-19 Pandemic.

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PHYTOCHEMICAL COMPONENTS, SUN PROTECTIVE PROPERTIES AND ANTIBACTERIAL ACTIVITY OF DESERT DATES (*Balanites aegyptiaca*) KERNEL OIL

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ABSTRACT

The current study aims to evaluate phytochemical compounds and investigate the in-vitro anti-microbial activities and sun protection factor of Desert dates (*Balanites aegyptiaca*) kernel oil collected in southern Algeria. A press oil machine was used to separate the crude oil from the seed oil. Classical spectrophotometric technique was used to assess the total phenolic compounds, flavonoids, as well as the chlorophylls and carotenoids. The oil's fatty acid composition was determined using GC-MS as FAME also the tocopherol compounds were quantified using HPLC-UV detector. *Balanites aegyptiaca* seed kernels are high in vegetable oil. The oil's phytochemical analysis revealed the presence of phenolic content (flavonoid content, saponin content, chlorophyll, and carotenoid content). The main fatty acids content were (palmitic acid 79.9%, oleic acid 54.4%, and linoleic acid). Also this oil is rich on tocopherol, including alpha tocopherol (580.8 mg/kg), Delta tocopherol (18.1 mg/kg). This same oil had good inhibitory activity against *Bacillus cereus* (11.2 mm), but no inhibitory activity against the other microorganisms tested was observed. The oil could be used as a skincare agent in cosmetic formulations, providing excellent sun protection which is regarded as an excellent source of biomolecules, can be used as a component in food or cosmetic products.

KEYWORDS:

Balanites aegyptiaca, seed oil, phytochemical compounds, GC/MS, Antimicrobial, sun protection factor

INTRODUCTION

Currently, research is being conducted everywhere in an effort to find an effective treatment for drug-resistant bacteria. The prospective drug will be

derived from natural products, according to the pharmaceuticals quest [1]. Moreover, the skin is the most regenerable organ in the body and functions as a barrier by managing thermogenesis, water balance, and permeability, it is thought to be a natural defense against microbial infections. However, a number of external factors can constantly harm this protective barrier biological harm and outside influences. For instance, prolonged solar radiation exposure without [2]. Since antiquity, aromatic and medicinal herbs have been employed for their therapeutic and medical benefits in various cultures. These benefits range from religious use to cosmeceutical and nutritional uses to medicinal benefits. Since these plants are sources rich in biologically active substances, primarily phenolic compounds, seed oils, much research has examined their usefulness in relation to their chemical composition [3].

Balanites aegyptiaca (L.) Delile (alob-Helgleg) family *Balantiaceae* is a wild tree grown in dry and savannah areas of Africa and south Asia [4]; which is well adapted to different agro-climatic regions with arid and semi-arid climatic features. Commonly it is known as 'desert date' [5]; in Algeria it is known as Togga; Tichet; Tabourag. The plant has also been shown to have hepatoprotective date' [6] [7], anthelmintic [8] [9], anti-inflammatory and analgesic [10], antiviral and antimicrobial, antioxidant properties [11].

The seed contains a lot of oil, protein, and minerals; additionally, the oil of *Balanites aegyptiaca* is very good and edible, with many fatty acids. It was also antimutagenic, antiviral, and antimicrobial against certain microorganisms.

Oil seed crops are cultivated primarily for edible oil. Oil seeds have received much interest due to increased demand for their healthy vegetable oils, livestock feeds, pharmaceuticals, biofuels, and other oleochemical industrial applications (Jimenez, Rahman). The suitability of oil for a particular application and purpose was determined by its characteristics, fatty acid and triglyceride compositions [12]. In addition to satisfying man's dietary needs, vegetable

oils are now widely used in bioresources and bio-diesel [13]. The aim of this study is to assess the chemical compounds of Desert dates oil and evaluate their antimicrobial and photoprotective activities .

MATERIALS AND METHODS

Chemical reagents. All chemical reagents were supplied by Sigma Aldrich.

Equipments. Press oil machine DL-ZYJ05, Spectrophotometer JENWAY, GCMS-TQ8040 NX,

Plants materials. The fruits of *Balanites aegyptiaca* were collected from Saoura region in southern Algeria; mechanical scraping of fruits to remove dry pulp; and recovering almonds after crushing the stones with a metal rod.

Test organisms. The strains used for the investigation were: *Escherichia coli* (ATCC 25922), *Sa-taphylococcus aureus* (ATCC 25923) *Pseudomonas aeruginosa*, (ATCC 27853), *Bacillus cereus*(ATCC 10876) ; *C. albicans* (ATCC 10231), *Acinetobacter baumannii* (ATCC19606), *Citrobacter freundii* (ATCC8090), *Enterococcus faecalis* (ATCC 29212).

Oil extraction: To separate the crude oil, the raw materials from the oil seed will be ground using a screw press oil machine, which will be separated by centrifugation and filtered.



FIGURE 01

Balanites aegyptiaca Fruits and seeds

Phytochemicals characteristics. Determination of extinction coefficients specific for K232 and K270. Extinction coefficients (K232 and K270)

were calculated using [14]. The application of ultra-violet absorbance coefficients provides information on the presence or absence of primary and secondary oxidation products in oil [15]. The higher the values of K232 and K270, the higher concentration of oxidation products in the oil. The extinction coefficient (K232 and K270) is the specific extinction of a 1% (w/v) oil in cyclohexane solution measured with a CARY 100 Varian UV spectrometer in a 1 cm cell path length.

Determination of chlorophyll and carotenoids content. The pigments (chlorophylls and carotenoids) were determined using the Minguez et al. method [16]. Using a spectrophotometer, the absorbance of a flask filled with 7.50 g of oil mixed with 25 ml of pure cyclohexane was measured relative to that of the solvent at 670 nm for chlorophylls and 470 nm for carotenoids. Spectrophotometer UV-Visible.

Classical spectrophotometric analysis of polyphenol content. Determination of Total Phenol Content. The concentration of total phenol content was determined using the method described by Slinkard and Singleton with slight modifications [17]. In brief, 0.25mL of plant extract was mixed with a diluted Folin-Ciocalteu reagent solution and vigorously shaken. After 3 minutes, 0.75 mL of 1% sodium carbonate solution was added to the mixture and allowed to react for 2 hours at room temperature. At 760 nm, the absorbance was measured. Using a gallic acid standard curve (1g GAE/mg), the total phenol content was expressed as mg gallic acid equivalents (GAE) per g of crude extract.

Determination of Total Flavonoid Content. The total flavonoid content was determined using the method described by Berk et al. [18]. 1mL of aluminum trichloride (2%) solution in methanol was mixed with 1mL of seed oil. After 10-minute incubation at room temperature, the absorbance of the mixture was measured at 415nm. Using a quercetin standard curve, the total flavonoid content was expressed as mg quercetin equivalents (QE) per gram of crude oil.

Saponin content. To make a decoction, a mixture of 2 g of plant sample in 100 mL of distilled water was brought to a boil for 30 minutes. The volume was re-adjusted to 100 mL after cooling and filtration. From this solution mother, 10 test tubes (1.3 cm internal diameter) with 1, 2, 10 mL were prepared from this solution. For all tubes, the final volume has been adjusted to 10 mL of distilled H₂O. For 15 seconds, each tube was vigorously shaken in a horizontal position. The height (in cm) of the persistent foam was raised after 15 minutes of rest in an upright position [19].

Tocopherols quantification. The tocopherols were quantified using a Waters High Performance Liquid Chromatography (HPLC) equipped with a UV detector at 280 nm by injecting a solution of 20 mg of oil in hexane and isopropanol (99:1) and filtering through a 0.45 mm filter. A polar capillary column (4.6 mm 25 cm) was used. The mobile phase contained 4% solution A and 96% solution B (A = 0.5 percent H₂PO₄ in water; B = acetone/acetonitrile, 50/50).

Dissolution of 20 mg of oil in hexane and isopropanol (99:1) and filtering through a 0.45 mm filter, capillary column (4.6 mm 25 cm) was used. The mobile phase contained 4% A and 96% B (A = 0.5 percent H₂PO₄ in water; B = acetone/acetonitrile, 50/50). The flow rate was set at 1.5 ml per minute. The wavelength of detection was 292 nm. Tocopherols were quantified using an external standard method and expressed in mg/kg [20].

GC/MS Analysis. The composition of fatty acids was determined in accordance with (EEC/2568, 2003) method [21]. Fatty acids (FAs) were converted to fatty acid methyl esters (FAMES) prior to analysis by trans-esterification of triglycerides with methanolic potassium hydroxide. Gas chromatography was used to analyze FAMES on an Agilent Technologies GC System chromatograph equipped with a FID. The injector was split, and the injected volume was 1 mL. The column used was an RT-2340 (60m0.25mm x 0.25 mm) type. Helium was used as the carrier gas, and the total gas flow rate was 1 mL/min. The initial and final column temperatures were 170 and 230 degrees Celsius, respectively, and the temperature was gradually increased at a rate of 4 degrees Celsius per minute. The injector and detector were both at 230 °C. As a result, the elution is performed in the order of increasing molecular weights.

Antimicrobial activity assay. Micro-organisms onto the surface of Muller Hinton agar, the broth culture of 24 h old test organisms was standardized using the 0.5 McFarland standards. The extract-impregnated discs were then placed on the

Muller Hinton at 5 mm spacing. Standard antibiotics were used as a positive control. The diameter of the inhibition zone was measured in millimeters after 24 hours of incubation [22].

Determination of MIC. The minimum inhibitory concentration (MIC) of oil of *B. aegyptiaca* was determined using 96-well microplate dilution assay as reported by Gulluce et al. [23] with slight modifications. One hundred microliter (100 µl) of Mueller-Hinton broth was loaded from the second to the twelfth test holes. A stock solution of the oil extract was prepared by dissolving 100 µl of the oil in DMSO and then adjusted to a final concentration of 100 mg/ml using Mueller-Hinton broth. To the first hole, 160 µl of the growth medium and 40 µl of the extract were loaded to reach a final concentration of 10 mg/ml.

Photoprotective Assay. Sun protection factor (SPF) measurement in vitro was used to evaluate the photoprotective activity of *Balanites* oil against UV damage. The sample was dissolved in pure methanol, and absorbance at 290-320 nm and 5 nm were measured. Using a UV spectrophotometer at periodicity (λ). The SPF was calculated as calculated using the formula below:

$$SPF = CF \sum_{290}^{320} EE(\lambda)I(\lambda)Abs(\lambda)$$

EE (λ) represents the spectrum of the erythral effect, I(λ) the spectrum of solar intensity, Abs(λ) the absorbance, and the correction factor (CF = 10). EE (λ) × I (λ) have constant values [24].

RESULTS AND DISCUSSIONS

The seed contain 29% of the oil that seem revealed strong presence of Total phenolic compounds (0.024± 0.22 EAG/g extract), total flavonoid content (0.53±0.01mg EQ/g extract) and saponin content were also present, while these phytochemicals may be responsible for the application of the oil in treating certain skin infections [25].

TABLE 01
Results of phytochemical characterization of desert dates seed oil

Parameter	Content
Oil yield	29%
Total phenolic content	0.024± 0.22 AGE/g extract
Total flavonoid content	0.53±0.01mg QE/g extract
Saponin content	+++
Extinction k232	0.04±0.01
Extinction K270	0.03±0.01
Cholorophylles mg/kg	2.41±0.61
Carotenoides mg/kg	2.54±0.23



FIGURE 02

Balanites aegyptiaca seed oil

Furthermore, Zang et al. noticed that the oil contained a high concentration of steroids. Carbohydrates, cardiac glycosides, and alkaloids were also found in the seed kernel oil, but anthraquinones, saponins, tannins, and flavonoids were absent in the seed oil of desert dates [13]. In order to better valorize seed oils, it was interesting to determine the content of total polyphenols. This family with its great structural has a beneficial effect on the diversity of health by protecting cells against free radicals, and it is also likely to be endowed with other pharmacological properties (antibacterial, anti-inflammatory, analgesic)[26].

The K values measured at 232 nm and 270 nm are associated with changes in the content of conjugated dienes and trienes formed due to polyunsaturated fatty acids oxidation. It is a measure of oxidation/rancidity and oil quality [27].

In general, K₂₃₂ levels rise due to improper fruit storage, outdated extraction or standardization methods. On the other hand, K₂₇₀ increases when the oil is not fresh and is the result of previous harvesting.

On the other hand, oil contained a substantial amount of chlorophyll (0.04 mg EQ/g extract). Other compounds found in the oils were comparable to chlorophylls and carotenoids, which are

involved in auto and photo-oxidation mechanisms [28]. They are in charge of the oil's color, which is an important factor in determining its quality.

TABLE 02
Tocopherol compounds of desert dates kernel oil

Vitamin E	Concentration mg/kg
Alpha-tocopherol	580.8
Delta-Tocopherol	18.1

As shown in Table 02, desert date oil has an alpha tocopherol content of approximately 580.8 mg/kg, followed by delta-tocopherol (18.1 mg/kg).

As mentioned by Elbadawia et al. [29], the total tocopherol content of the boiled and roasted oil samples was found to be 42.2 mg/100g, 39.85 mg/100g, and 40.05 mg/100g, respectively. *Balanites aegyptiaca* oil contains more tocopherols than coconut oil, refined olive oil, and extra virgin olive oil [29]. Tocopherols are biologically active antioxidants found in nature [30]. They regulate the oxidative stability of the oil and protect the fatty acids by removing free radicals and reactive oxygen species [31].

Table 03 shows the GC-MS results and identities of the fatty acids present in the seed kernel oil of *Balanites aegyptiaca*. The most prominent of the fatty acids are Oleic acid (54.4%), linoleic acid (29.5%), Palmitic acid (21.9%), Stearic acid (11.77%), oleate glycidyl (4.4%). These results are comparable to those of Zhang et al. [13], who found palmitic acid (20.51%), oleic acid (28.32%), linoleic acid (19.20%), and stearic acid (15.97%). The oil contains about 47.52% unsaturated fatty acid, which makes it a good edible oil [13]. Oleic acid of 43-49.1% and linoleic acid of 29-36% are the reference levels for the fatty acid content, which is a crucial indicator of the nutritional value of oils [26].

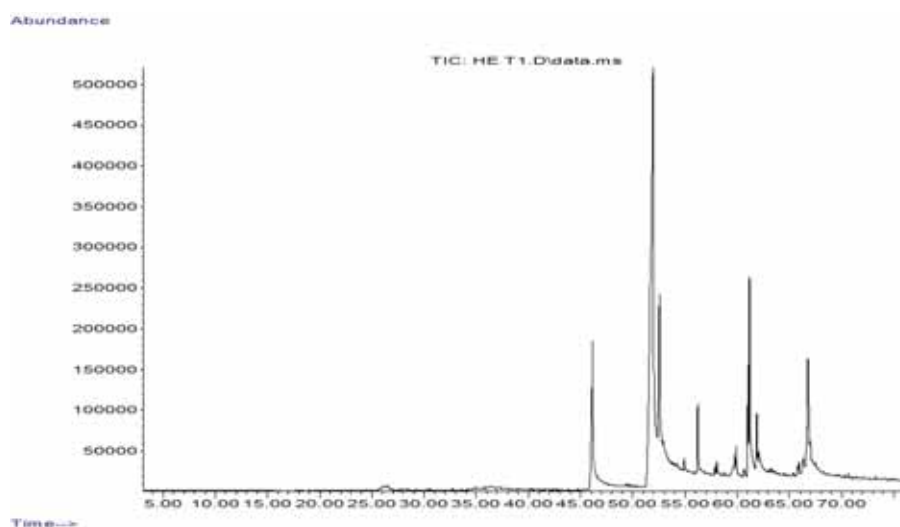


FIGURE 03

Fatty acid profile of desert dates kernel oil

TABLE 3
Fatty Acid compounds of desert dates kernel oil

Fatty acids	Percentage
N Hexadécanoïque acide	Palmetic acid 21.9%
9,12- Ooctadécadénoïque Acid	Linoleic acid 29.15%
	Oleic acid 54.4%
Oleate Glycidyl	4.4%
2-Oleoylglycerol, 9-Octadecenoic acid, 2,3-dihydroxymethyl-, ethyl ester	Stearic acid 11.77%

TABLE N 03
Triglycerides compounds of Desert dates (*Balanites aegyptiaca*) seed oil

Triglycerides	Percentage(%)
PPPI	0.22
PILL	4.22
POL	25.87
SOL	55.13
SAO	0.1

"P: Palmitic acid C16; Pl: Palmitolic acid C16; L: Linoleic acid C18; O: Oleic acid C18; S: Stearic acid C18; A: Arachidic acid C20.

TABLE 04
Results of an antimicrobial susceptibility test performed on Oil and

	Concentrations mg/ml					CZ	CMI	CMB
	500	250	125	62.5	31.25			
<i>Sataphylococcus aureus</i>	–	–	–	–	–	18mm	++	++
<i>Escherirchia coli</i>	6.5mm	–	–	–	–	19mm	++	++
<i>Pseudomonas aeruginosa</i>	–	–	–	–	–	17 mm	++	++
<i>Enterococcus faecalis</i>	–	–	–	–	–	23mm	++	++
<i>Acinetobacter baumannii</i>	–	–	–	–	–	17mm	++	++
<i>Citrobacter freundii</i>	6.2mm	–	–	–	–	19,3mm	++	++
<i>Bacillus Cereus</i>	11.2 mm	8.5mm	6 mm	–	–	31mm	250mg/ml	++
<i>Candida Albicans</i>	–	–	–	–	–	–	++	++

As shown in the Table 03 Our oils' examination also enabled the identification of five triglyceride-related chemicals, the majority of which are PPPI, PILL, POL, SOL, and SAO. These are lipid compounds made from a glycerol molecule esterified by three occasionally different fatty acid molecules [32]. *Balanites aegyptica* is considered in this research is organic oil, a triglyceride and is generally referred to as vegetable oil [13].

Results on the antimicrobial activity of the oil are shown in the Table 04.

The extracted oil inhibited the tested bacteria to varying degrees (Table 04), and the results were expressed in terms of the diameter of the growth-inhibition zone (clear zones). The oil had significant antibacterial activity against *Bacillus cereus* (11.2 mm) but low antimicrobial activity against *Escherichia coli* and *Citrobacter freundii* (6.5 mm and 6.2 mm, respectively). The results show that *Balanites* oil has

no inhibitory effect on the other microorganisms tested.

Daya and Vaghasiya [25] demonstrated the use of this oil to treat some bacteria, and the lack of inhibition effect observed in the evaluation could be attributed to variations in the oil extraction process. Elfeel reported differences in the properties of *Balanites aegyptiaca* seed kernel oil [33].

Sun protection factor. *Balanites* oil has been found to have a significant capacity to absorb UV rays (SPF: 11.50±0.10). Oil is the most significant component of creams and lotions, so studying the SPF values of non-volatile oils will help in the selection of oils for the formulation of various cosmetic dosage forms. The sun protection factor (SPF), which mainly reflects UVB protection, is a quantitative measure [34]. SPF is generally divided into four

TABLE N 05
SPF values of *Balanites* oil

CFx EE(λ)xI(λ)x Ab (λ)1	CFx EE(λ)xI(λ)x Ab (λ)2	CFx EE(λ)xI(λ)x Ab (λ)3		
0.19425	0.1926	0.19605		
0.86602	0.963243	0.957524		
3.414312	3.313722	2.98896		
3.85165	3.83526	3.717252		
2.20884	2.151056	2.108184		
0.974918	0.944714	1.62766		
0	0	0	Moyenne	SD
11.51	11.40	11.60	11.50	0.10

groups: low protection (SPF value between 2 and 15), high medium protection (FSP value between 15 and 30), high protection (FSP value between 30 and 50), and very high protection (FSP value greater than 50) [35]. Based on the results of this work, there is a strong correlation between total phenolic contents and the photoprotective activity of *Balanites* oil. According to the literature, these compounds are considered excellent sun filters with significant photoprotective effects. Additionally, the presence of cyclic and aromatic hydrocarbons gives them the capacity to absorb UV light with wavelengths of between 240–285 nm and 300–550 nm [24].

CONCLUSION

The present work was based on the phytochemical content and antimicrobial screening of oil extracted from *Balanites aegyptiaca* seeds. We observed that the seed of this plant contains a significant amount of virgin oil that seem content phenolic compounds, flavonoids and saponin. Linoleic, oleic and palmitic acids were predominant which are important in cognition and motor activity and have beneficial physiological effects in the prevention of cardiovascular diseases and cancer. In addition, this oil is rich on tocopherol content an interesting nutritional composition. An interesting nutritional composition characterized tested oil. This technology preserves the chemical composition of the oil and can be used successfully as a source of dietary oil for human consumption. The oil could also be a great source of biomolecules. Overall, in this research, *Balanites* oil proved to be the most effective candidate as natural preservatives to be used as sources of health-promoting compounds it providing inhibition against *Bacillus cereus* and sun protection, also the oil could be used as a skincare agent in cosmetic formulations, providing excellent sun protection. Finally, the oil, a fantastic source of biomolecules, can be utilized in cosmetics compositions or as food additives. It is a smart way to contribute to the preservation and survival as well as the sustainable development of the critically endangered Algerian desert date tree, to use this oil to enrich the production of bioactive components with high value in food and therapy and desert date tree to use this oil to enrich

the production of bioactive components with high value in food and therapy and to help increase the profitability of desert date tree cultures.

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ANTIFUNGAL ACTIVITY OF TAR *Citrullus Colocynthis* SEEDS AGAINST *Fusarium Oxysporum F. Sp Albedinis* THE CAUSAL AGENT OF THE BAYOUD DISEASE AND GAS CHROMATOGRAPHY-MASS SPECTROMETRY ANALYSIS

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ABSTRACT

The most dangerous disease of date palms in Algeria is called bayoud, which is a vascular disease caused by *Fusarium oxysporum f. sp. albedinis* (Foa).

The objective of this work is the isolation of *Fusarium oxysporum f. sp. albedinis*, from different oases in southwestern Algeria Foa strains, are identified by PCR method analysis then the tar extraction of the *Citrullus colocynthis* seeds and evaluate the antifungal activity by using the direct contact method of tar *Citrullus colocynthis* seeds and evaluate the antifungal activity of their fractions of the dichloromethane extract by using the broth microdilution method then analyze active fraction by gas chromatography-mass spectrometry.

Citrullus colocynthis seeds tar effectively inhibited Foa growth in vitro with a minimum inhibitory concentration of 2.10 µg/ml and fraction A (0.512 mg/ml), GC-MS was identified as 21 components.

These consequences are very encouraging for using this extract in the field on diseased date palms to limit the damage caused by this fungus.

KEYWORDS:

Antifungal activity, Bayoud, *Fusarium oxysporum f. sp. albedinis*, *Citrullus colocynthis* tar, GC-MS

INTRODUCTION

Bayoud is an Algerian name for phytopathogenic fungi that infect various plants, mainly the date palm, and is resilient to abiotic conditions [1]. Bayoud is a fungal disease of the trachea caused by the soil-borne fungal pathogen Foa. Parasites attack palm trees through the roots and eventually colonize the entire vascular system, causing the date palm to

wither and eventually die [2]. Recent trends favor biological means of control as an alternative way to avoid environmental pollution by reducing the intensive use of pesticides [3]. These encourage researchers to discover, develop and synthesize new efficient, active, and less toxic molecules for systemic activities [4]. *Citrullus colocynthis* belongs to the family of *Cucurbitaceae* it is distinguished by the occurrence of many constituents such as flavonoids, alkaloids, carbohydrates, tannins, gums, and mucilage [5]. To the best of our knowledge, there are no previous reports on the tar from *Citrullus colocynthis* seeds Thus, as a continuation of our antifungal studies of medicinal plants in the Algerian Sahara, we found that tar from *Citrullus colocynthis* seeds had an inhibitory effect on *Fusarium. oxysporum f. sp. albedinis* (Foa), It results in Bayoud on the date palm in the Algerian Sahara.

The objective of this work is the isolation of *Fusarium oxysporum f. sp. albedinis*, from different oases in southwestern Algeria and Foa strains are identified by PCR method analysis and evaluate the antifungal activity of the fractions (A, B, and C) of the dichloromethane extract of tar *Citrullus colocynthis* seeds then analyze active fraction by gas chromatography-mass spectrometry (GC -MS).

MATERIALS AND METHODS

Extraction of tar: *Citrullus colocynthis* seeds were collected in October 2021 in the Bechar region of southwestern Algeria, a modified fractional distillation method was used to remove the tar for six hours. Distilled tar was stored at room temperature until analysis [6].

Fraction of the dichloromethane extract: Mix 10 g tar of the *Citrullus colocynthis* seeds with 10 g of gel silicate in a glass Petri dish, then smooth the dried powder away from humidity and light for

24 hours. Then we use Vacuum filtration, then pouring 500ml dichloromethane A series of filtration and evaporation was carried out for the recovered solvent (three times) [7], and 2 g of the extract obtained was fractionated on a column containing silica gel using non-polar phase (basic): Benzene/Ethanol/Ammonia (90:10:1) [6].

Fungal isolate: *Fusarium oxysporum f. sp. albedinis* strains were isolated from date palm rachis with symptomatic Bayoud, which were obtained in southwest Algeria according to the protocol of Djerbi (1990) [8], then were identified by PCR method [1].

***Fusarium oxysporum f. sp. albedinis* PCR analysis:** For DNA extraction we employed the CTAB technique described by Nunes *et al* (2011) [9,10]. PCR primers for amplification of *Foa* included two primer pairs: *FOA1* (CAGTTTATTAGAAATGCCGCC) coupled with *BIO3* (GGCGATCTTGATTGTATTTGGTG) and *FOA28* (ATCCCCGTAAGCCCTGAAGC) coupled with *TL3* (GGTCGT CCGCAGAGTATACCGGC). The PCR reactions were performed in a total volume of 25 μ l, containing 1 μ l of genomic DNA (~100 ng), 1.5 mM MgCl₂, 25 mM dNTP, 0.5 U Taq DNA polymerase (Biomatic), 2.5 μ l 10 \times reaction buffer, 1 μ l DMSO, 17 μ l ultra-pure water, and 0.5 μ M of each primer. Amplification was performed using a GTC96S Thermal Cycler (Clever), we used the amplification program described by Fernandez *et al* (1998) [11].

Antifungal activity of tar *Citrullus Colocynthis* seeds: Antifungal activity was determined by the method of contact direct as described by Bhutani *et al* (2018) [12], we obtained the following Concentrations (0.25, 0.51, 1.04, 1.30, 1.57, 1.83, 2.10, 2.38 and 2.65 μ g/ml) respectively, [13], recommended, additionally, a control assay was employed, and three copies of each test were run. [14].

Statistical analyses: All the measurements were made in triplicate and the results obtained were expressed as the mean \pm standard deviation (SD). One-way ANOVA was carried out to test for any significant difference. Differences between means at $P \leq 0.05$ level were considered significant.

Antifungal activity fraction of the dichloromethane extract of tar *Citrullus Colocynthis* seeds by broth microdilution method: For the evaluation of the antifungal activity of the fractions, the modified Broth microdilution technique was used, with an inoculum concentration of 10⁶ spores per ml, and a PDB medium. The final concentrations of the antifungal agents were 4.09mg/ml for fractions A, B, and C to 0.510 mg/ml incubated for 3

days at 25°C. The sterile control (PDB), and the negative control (PDB and inoculum). The plates were incubated at 30°C after 3 days to add 25 μ l of 0.3 M para-iodonitrotetrazolium (INT) to each well [15]. Followed by incubation at 30°C for 2 hours. The positive result was defined as no appearance of a pink color after adding INT [16].

Chemical composition: GC parameters were as follows: The carrier gas and make-up gas were high pure helium and nitrogen, respectively. The carrier gas (helium) was set at a flow rate of 1.0 mL \cdot min⁻¹. The inlet temperature was 200 °C with a split ratio of 10:1 and the pressure was 11.6 psi. The column oven temperature was initially set at 60 °C for 5 min, and then ramped to 200 °C at 5 °C \cdot min⁻¹ for 5 min, and after that, it was warmed up to 300 °C at 10 °C \cdot min⁻¹.

MS parameters were as follows: Data were acquired in the electron impact (EI) mode, using the full scan mode from m/z 30 to 600 at 1562 amu/s. The ion source temperature and quadrupole temperature were 230 and 150 °C, respectively. The identification of volatile compounds was based on a comparison of their GC retention time and mass spectra with the retention index of n-alkane-saturated alkanes and the reference spectra from the US National Institute of Standards and Technology [17]. The values were the mean of three replicates of each sample. Data were analyzed by using Agilent Mass Hunter Analysis.

RESULTS AND DISCUSSION

Physicochemical properties of *Citrullus colocynthis* grains tar: Tar of *Citrullus colocynthis* seeds is a dark brown, viscous liquid with a smoky odor, the results of the physical and chemical of vegetable tar are shown in Table 1 with the yield of 6.72 % (v/w) on dry weight basis. It has a pH of 5.5 , a refractive index of 1.4850 ,Specific density at 20 °C is 0.77 and rate of dry water is 50%.

Is very slightly soluble in water; soluble in ether, chloroform, amyl alcohol, ethyl acetate ,alcohol; and partly soluble in petroleum ether..

Our results are very close to those reported by Benlarbi (2019) [6]

Isolation and morphology of FOA strains: The FOA strains were isolated from the rachis of contaminated cultivar palms in the southwest Algeria palm grove (Figures 1). The FOA isolates revealed minor macroscopic morphological variability, such as the color of the mycelium as observed by Benabbes *et al* (2015) [18]. The color of the colonies and the appearance of the mycelium obtained from the first subculture are typical of the wild form of F.o.a. and conform to those described by Benlarbi (2019) [6].

TABLE 1
Physicochemical characterization of vegetable tar of *Citrullus colocynthis* grains

Rate of dry water	Specific density at 20 °C	Refractive index	PH
50%	0.77	1.4850	5.5

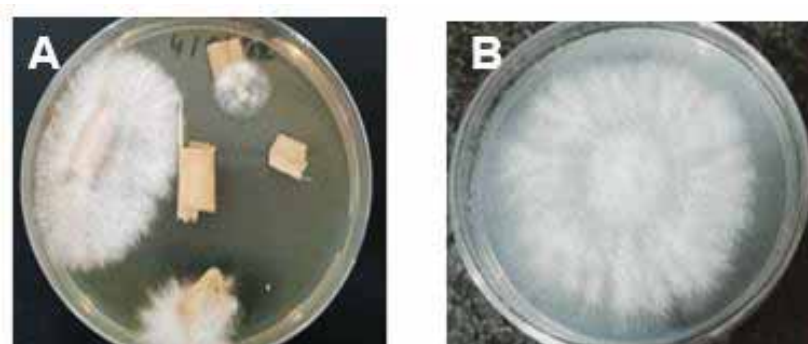


FIGURE 1
A.B isolation of *FOA* in PDA medium. (Original Source, 2021).



FIGURE 2
***Fusarium oxysporum* f. sp. *albedinis* species-specific PCR amplification (Original Source, 2021).**

TABLE 2
Antifungal activity of *Citrullus colocynthis* tar against FOA isolates using radial growth contact direct method

$\mu\text{g/ml}$	Control	0.25	0.51	1.04	1.30	1.57	1.83	2.10	2.38	2.65
Foa1	60,13±0,35	38,6±0,52	31,06±0,11	24,16±1,35	21,83±0,37	12,9±0,36	6,03±0,05	3±0	0,06±0,11	0
Foa 8	56,86±0,75	36,16±0,28	30,16±0,28	26,16±0,28	22,83±0,8	12,13±0,23	8±0,01	2,96±0,05	0	0
Foa 9	60,16±0,28	40,03±0,55	34,4±0,25	30,5±0,43	25,16±0,28	15±0,3	7,96±0,15	4,93±0,05	0	0
Foa 15	59,03±1,07	39,03±0,04	30,36±0,47	24,86±0,23	22,83±0,25	10,6±0,52	6,16±0,15	3,03±0,05	0	0
Foa 18	59±0,3	43,93±5,3	42,13±0,32	34,96±0,05	22±0,63	14,26±0,40	7,35±1,41	2,07±0,58	0	0
Foa 31	54,06±0,20	45,13±0,41	35,16±0,76	25,03±0,5	21,9±0,1	14,16±0,20	7,39±0,11	2,3±0,26	0	0
Foa 54	58±0,2	45,9±0,46	40,33±0,30	35,1±0,36	22,96±0,25	17,2±0,20	7,03±0,05	0	0	0
Foa 63	61,5±0,5	57,96±0,04	54,06±0,40	49,8±0,52	39,43±0,66	25,03±0,05	14,8±0,26	7,13±0,15	2,9±0,1	0
Foa 64	74,5±0,5	60,1±0,55	54,93±0,60	51,33±0,88	39,03±0,15	27,7±0,26	17,03±0,05	9,03±0,05	1,9±0,1	0

Data are presented as means \pm SDM (n=3), $P \leq 0.05$.

TABLE 3
Difference between groups (ANOVA)

	F	P-Value	F crit
FOA1	4485.18867	4.845E-31	2.39281411
FOA8	9883.02832	1.802E-34	2.39281411
FOA9	14378.7868	4.2445E-36	2.39281411
FOA15	16787.0495	9.0247E-37	2.39281411
FOA18	538.964404	7.3583E-22	2.39281411
FOA31	9323.59378	3.2265E-34	2.39281411
FOA54	23443.8643	3.1996E-38	2.39281411
FOA63	13988.3925	5.5892E-36	2.39281411
FOA64	8761.66213	6.0065E-34	2.39281411

TABLE 4
Antifungal activities against fractions A, B, and C against FOA isolates by broth microdilution method

	Fraction A	Fraction B	Fraction C
(mg/ml)	0.512	2.048	2.048

PCR assays for specific identification of *Foa*.

To identify FOA pathogenic isolates, specific PCR assay primer pairs TL3-FOA28 and FOA1-BIO3 were used. Using this specific PCR assay, 400 pb and 204 pb fragments were detected, and the strains we isolated from infected date palms were identified as the FOA Bayoud pathogen (Figure 2).

Antifungal activity and MIC determination.

The antifungal activity by the direct method of the *Citrullus colocynthis* grains tar exhibited significantly high antifungal activity against FOA (Table 2). The vegetable tar induced a great inhibition over the mycelial growth of strains of FOA, from the concentration 2,1µg/ml to 2.65µg/ml. We found that our *Citrullus colocynthis* grains tar extract inhibited Foa growth on the PDA medium indicating the presence of potent antifungal compounds in our extract. Similar antifungal activity of tar by studies by Bendjima et al (2020) [19], Terfaya et al (2019) [20], Gumgumjee (2020) [21], and Benlarbi et al (2014) [22]. P-value < 0.05 (significance level) so there is a significant difference between the groups (09 strains) that is to say there is an influence of the concentration on the champions (Table 3).

Antifungal activity fraction of the dichloromethane extract of tar *Citrullus Colocynthis* seeds by broth microdilution method was investigated for their antifungal activities against nine strains of Foa Its sensitivity to mentioned fraction A is 0.512 mg/ml in all Foa and 2.048 for fractions B and C (Table 4). In comparison between the different plant extracts (crude tar, chromatographic fractions), crude tar manifests a more interesting inhibiting power than that of others. This fungi toxicity is probably due to the quality of the molecules of the extract tested, given their pungent smell. This observation confirms by Terfaya (2020) [23].

Identification of chemical compounds in Fraction A by gas chromatography-mass spectrometry.

The results of GC-MS analysis of fraction A led to the determination of several biological compounds as presented in Table and Figure 3, the fraction A of dichloromethane extract of *Citrullus colocynthis* tar leaves showed the presence of twenty-one compounds however, acid palmitic (10.38%), Heptacosane-1-ol (9.58%), tetradecane (9.45%), 4-(1-methyl) ethoxy-1-butanol; 5-methoxy (8.47%), Isothymol (8.38%), acid stéarique (7.38%), acid mystique (6.25%) were the major compounds, and other minor compounds were present in low quantities, with relative peak areas ranging from 5.91% and 2.03%. These studies confirm the results of Gupta AS and Chakrabarty MM, (1964) [24].

CONCLUSION

The results of Antifungal activity have been determined to have an effect against *Fusarium oxysporum f. sp. albedinis*, with a high recorded activity of tar grains of *Citrullus colocynthis*. These results are very promising for practicing this extract in the field on diseased date palms to limit the damage caused by this fungus, by in vivo tests carried out on the spot and controlled, aiming at elaborating a biopesticide, is necessary, on one hand, to confirm the results we have obtained and, on another hand, to study their potential efficacy under different. In summary, an extracted fraction of dichloromethane of the grains of the *Citrullus colocynthis* was evaluated for its antifungal activities. acid palmitic, Heptacosane-1-ol, and tetradecane were identified as the major compounds in fraction A.

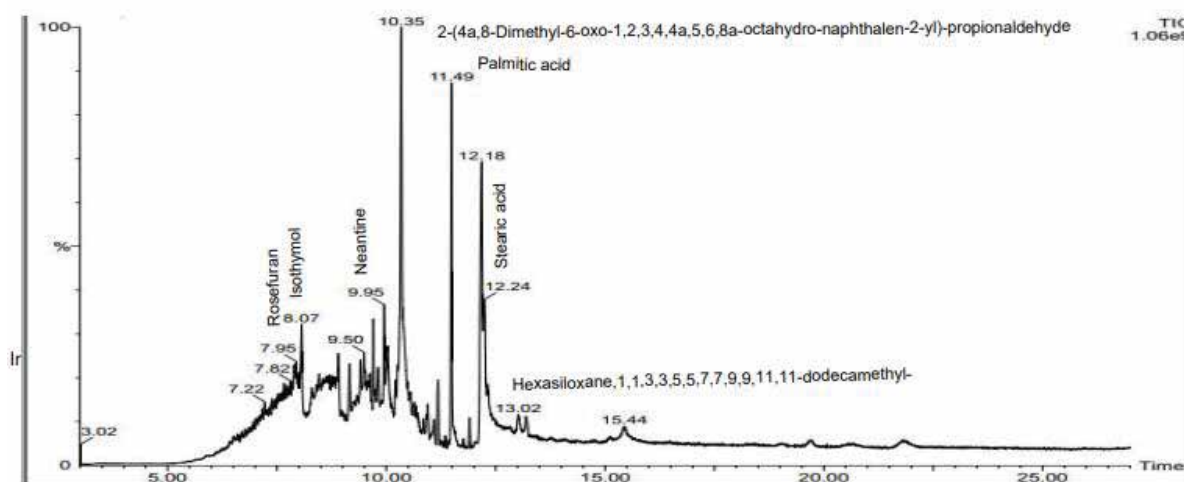


FIGURE 3

GC-MS chromatograms of dichloromethane fraction A (Original Source, 2022).

TABLE 5
Chemical composition of the GC-MS analysis of fraction A of dichloromethane extract of *Citrullus colocynthis* tar.

#	RT	Scan	Height	Area	Amount %	Name
1	7.95	160	147,465,616	14888766	5.45	Rosefuran
2	8.07	365	149,535,424	22912712	8.38	Isothymol
3	9.01	925	524,748,320	16161104	5.91	Neantine
4	9.22	1305	419,595,808	79906608	5.24	2-(4a,8-Dimethyl-6-oxo-1,2,3,4,4a,5,6,8a-octahydro-naphthalen-2-yl)-propionaldehyde
5	9.45	1995	360,477,856	27821900	10.18	acid palmitique
6	9.65	2207	237,080,560	20167756	7.38	acid stéarique
7	9.84	1995	360,477,856	8796114	3.22	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-
8	10.22	1260	28,701,974.0	235641	2.03	a oléique
9	10.35	125	46,301,720.0	354227	4.01	acid linoléique
10	10.42	585	43,723,716.0	276740	6.25	Acids myristique
11	10.63	547	25,949,804.0	276740	5.63	3,4-dimethyl,2-hexanone; 2- methyl 4-heptanone ; 3-Methyl,2-heptanone
12	10.96	1945	21,773,380.0	323460	4.65	1-propoxypentanecid ; 2,3-epoxymethyl propionate
13	11.15	458	43,283,424.0	35370	3.95	Trimethylsilylmethanol
14	11.33	654	615,138,752.0	21354	6.45	benzenedicarboxylicacid
15	12.02	254	35,387,120.0	21354	9.58	Heptacosane-1-ol
16	12.51	365	28,967,844.0	213546	4.15	2-furanmethanol,tetrahydro-5-methyl-
17	12.69	564	455,984,928.0	443338	5.12	5-methoxy,2-methyl,2-pentanol
4	12.82	1305	615,138,752.0	35370	8.47	4-(1-methyl)ethoxy-1-butanol; 5-methoxy
5	12.96	524	35,387,120.0	213546	9.45	tetradecane
6	13.02	965	28,967,844.0	21354	5.26	hexadecane
7	15.44	245	455,984,928.0	21354	2.22	2-hexanone

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Materials and methods:

Please be as precise as possible to enable other scientists to repeat the work.

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Acknowledgements: Acknowledgements of financial support, advice or other kind of assistance should be given at the end of the text under the heading "Acknowledgements". The names of funding organisations should be written in full.

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Discussion and Conclusion: This part should interpret the results in reference to the problem outlined in the introduction and of related observations by the author/s or others. Implications for further studies or application may be discussed. A conclusion should be added if results and discussion are combined.

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