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Biochemical properties and antimicrobial activity of the pods extract of Sudanese carob (*Ceratonia silique L.*)

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KEYWORDS

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ABSTRACT

This study was carried out to determine the phytochemical profile and antimicrobial activity of the carob, *Ceratonia silique L*., (Cs). Mature fruits of Cs trees were randomly collected, and dried in grounded into fine powder. Phytochemical, amino acids, and antimicrobial activity analyses were then applied. The Cs fruits displayed high amounts of terpenoids and moderate amounts of flavonoids and steroids. The pods contained seventeen amino acids, including the nine essential amino acids (histidine, valine, threonine, lysine, isoleucine, leucine, phenylalanine, arginine, and methionine). The remaining eight amino acids were nonessential. Serine displayed a low level (0.63 µg/ml), whereas proline was the most abundant amino acid (232.38 µg/ml). The ethanol extracts of CS pods at a concentration of 100% displayed noticeable activity against the microorganisms utilized in this study (*Bacillus subtilis, Staphylococcus aureus, Pseudomonas, Klebsiella pneumonia, Streptococcus, and Escherichia coli*). Active inhibition was also observed when using 80% concentration for all organisms except *Escherichia coli, Pseudomonas,* and *Bacillus subtilis*. It is concluded that the extracts of Cs can be considered a good source of amino acids. The plant also had significant antibacterial activity.

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INTRODUCTION

Carob is used in many Arab countries, including Sudan, to make a popular drink that is consumed all over the year. Carob is also used as a medicinal plant and preparation of special traditional types of Arabic confectionery. In western countries, carob powder is produced by deseeding carob pods, yielding kibbled carob, followed by roasting and milling of the kibbled carob. Carob juice concentrate is produced by boiling carob juices without any added ingredients and technological or scientific techniques. Due to its high sugar content, carob was consumed as a food, especially in ancient times, as a sweet for children or in emergencies such as wars (Owen *et al*., 2005). The carob (*Ceratonia siliqua L.*) is thought to be a tropical plant that has adapted well to Mediterranean climates by utilizing its deep rooting habit and xerophilous leaves to avoid water stress (Catarino, 1993).

Both fruit and pulp of the carob consist of a wrapping regular seed. Indeed, the sweet pulp of the carob has been used as cattle feed for a long time (Ait *et al*., 2007). In addition, fruits and leaves of the carob tree contain proteins and phenolic compounds, and a wide range of physiological activities that include antioxidant, antimutagenic, anticarcinogenic, and anti-inflammatory properties (Custódio *et al*., 2011). Due to their some interesting properties, the pulps, and the seeds have been also used in the pharmacological industry (Makris and Kefalas, 2004). Furthermore, in recent years, carob has been used in the food industry as biomass substrate and thus has attracted the attention of producers because of its increasing market value.

Medicinal and aromatic plants are some of the most prominent sources of phenolic compounds, and their phytochemical constituents often have therapeutic value or can be precursors for the development of pharmaceuticals. They have been used worldwide since prehistoric times for treating several ailments and are still find increasing use today in herbal medicine formulations as well as alternative and complementary medicine. Indeed, medicinal plants are still widely used in ayurvedic and Chinese traditional medicines (Jain *et al*., 2019). Generally, plants are known to produce certain chemicals which are naturally toxic to bacteria, and a large body of literature has validated the antimicrobial activity of plant extracts showing great potential, especially against multidrug-resistant bacteria (Pesewu *et al*., 2008; Tasdelen *et al.,* 2009). It is known that many *Ceratonia siliqua L*. extracts have biological activity, in vitro and in vivo, which justified the continuity of research on the characterization of antimicrobial activity of these plants (Martinez *et al*., 1996). Edible pods are not only used as fodder for breeding cattle, but they are also well known as a source of health products.

Ceratonia silique L. (Cs) grows naturally in Western Kordufan, Sudan, where it is considered by the locals as the most important medicinal plant in the area (Al Shafei *el al.,* 2016). The study aimed to investigate the effect of *Ceratonia silique L.* pods extract on some microorganisms and identify the phenolic compounds and types of amino acid content.

MATERIAL AND METHODS

Plant materials

Mature fruits of Cs trees were used in this study. The fruits were randomly collected from the trees grown in the vicinity of Ghubaysh village, Western Kordufan State, Sudan (Fig. 1). The fruits samples were cleaned carefully, and freed from stones, dirt, and other materials. The fruits were dried in the shade and subsequently grounded into fine powder using mechanical blender. The powder was then packed in airtight containers with proper labeling, and kept for use in the future.

Phytochemical analysis

Test for saponin, tannins and steroids

For saponins, the powder of Cs fruits was mixed with 5 ml of distilled water in a test tube and then shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins. For tannins, the powder was mixed with

2 ml of 2% solution of FeCl3. A blue-green or black coloration indicated the presence of tannins (Yadav and Agarwala, 2011). For steroids, Cs fruits powder was mixed with 2 ml of chloroform and concentrated sulphuric acid was added sidewise. A red color produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then, 2 ml of concentrated sulphuric acid, 2 ml of acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids (Yadav and Agarwala, 2011).

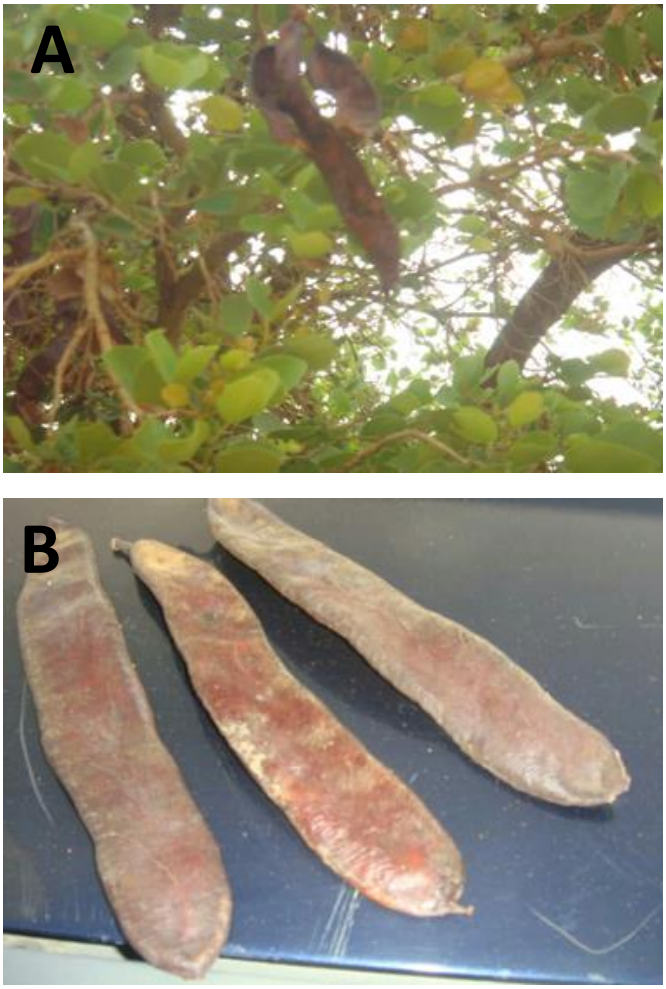

Fig. 1: Photographs showing carob (*Ceratonia silique*) tree (A) and its fruits (B).

Test for flavonoids

The detection of flavonoids in the Cs fruits powder was applied using 0.5 g of plant extract. Briefly, the extract was placed in a test tube, and 10 ml of distilled water were added. Five ml of dilute ammonia solution were added to a portion of the aqueous filtrate of the plant extract followed by addition of 1 ml concentrated sulphuric acid. The presence of flavonoids in each extract was indicated by yellow color (Yadav and Agarwala, 2011).

Test for terpenoids

An amount of 0.8 gram of the Cs powder was placed in a test tube, then 10 ml of methanol was added, and the tube was shaken well. The solution was then filtered. Five ml of the plant extract of plant sample was taken. Then 2 ml of chloroform were mixed with the extract and 3 ml of sulphuric acid were added. The formation of reddish-brown color indicated the presence of terpenoids (Wadood *et al*., 2013).

The presence of phytochemicals in Cs extract was evaluated semi quantitatively by the colour intensity indicated by four scales: low +; Small ++; Moderate +++; High ++++ (Table 1).

Total amino acids analysis

One gram of dried leaves sample was transferred into a bottle with a screw cap, and then 25 ml of hydrolysis mixture were added and mixed. Bottles containing the mixtures were placed into an oven set at 110 ºC for one hour. The bottles were then closed and left in the oven for 24 hrs. After hydrolysis, the bottles were removed from the oven, cooled down to room temperature, and their caps were carefully opened. Two ml of the internal standard solution (Norleucine) was added to the hydrolyte and mixed. By using a rotary evaporator, the volume was reduced to 5-10ml. under vacuum at 60ºC. The pH was adjusted to 2.2 with sodium hydroxide solution and make up to 50ml with citrate buffer. Quantitatively transferred pH was adjusted with citrate buffer to 200 ml in a volumetric flask, and made up to 200ml with citrate buffer. A suitable amount was filtered through a 0.2 µm membrane filter. The filtrate was analyzed using automatic amino acid analyzer.

Plant extraction for antimicrobial analysis

Ethanol extraction

Twenty-five ml of ethanol and 1 gram of plant powder was added to a beaker. The beaker was covered and shaken well every 10 minutes for 1 hour, and then kept at room temperature for 24 hrs. The solution was filtered through a filter paper and the filtrate was concentrated in a water bath at 45ºC, after which the extract was transferred into a sample bottle and stored in a refrigerator for further analysis.

Antimicrobial activity

Six different species of bacteria were used for the analysis of the antimicrobial activity of Cs extracts. The pure isolates of these bacteria were obtained from the Department of Microbiology, College of Veterinary Medicine, University of Bahri, Sudan. The isolates include: *Bacillus subtilis, Staphylococcus aureus, Pseudomonas, Klebsiella pneumonia, Streptococcus and Escherichia coli*.

The agar diffusion method was used to examine the antimicrobial activity study. Sterile nutrient agar powder was prepared by dissolving 28 grams of the agar powder in one liter of distilled water, which was boiled to ensure complete dissolution. The nutrient agar was then sterilized at 121ºC for 15 minutes, dispensed into labeled petri dishes, and allowed to gel. Twenty ml of sterile molten nutrient agar in a petri dish was seeded with 1.0 ml. of standardized broth cultures of the bacteria. Six mm diameter wells were bored into the agar with sterile cock pourer and filled with 0.4 ml of various dilutions of the extracts: 40%, 60%, 80% and 100% in distilled water. The petri dishes were incubated at 37ºC for 24 hrs. At the end of incubation, the zones of inhibition that developed were measured in millimeter (mm) with the help of a transparent ruler. Distilled water was used as negative control, and ciprofloxacin antibiotic was used as a positive one. Diameter of zones of inhibition ≥10 mm exhibited by plant extract was considered active.

RESULTS

Phytochemical analysis

The different phytochemical components of the Cs extract are shown in Table 1. The plant contained high amounts of terpenoids, and moderate amounts of flavonoids and steroids.

Amino acids content of Ceratonia siliqua L pods

Tables 2 and 3 show the essential and non-essential amino acid content of *Ceratonia silique L.* pods, respectively.

Seventeen different types of amino acids in Cs pods were detected. The nine essential amino acids (histidine, valine, threonine, lysine, isoleucine, leucine, phenylalanine, arginine, and methionine) were shown in table 2, whereas the rest were nonessential amino acids which are presented in Table 3. Apart from serine (0.63 µg\ml), the content of the other amino acids was remarkably higher. Proline (232.38 µg/ml).

Table 1: The intensity of phytochemical component of *Ceratonia siliqua L*. pods extract.

Component	Intensity
Tannins	++
Steroids	+++
Flavonoids	+++
Terpenoids	++++
Saponins	+

Table 2: Essential amino acids content of *Ceratonia silique L.* pods.

N0.	Amino acid	Amount (µg/ml)
1	Histidine	3.09
2	Valine	33.81
3	Leucine	14.84
4	Isoleucine	15.22
5	Lysine	5.89
6	Threonine	4.42
7	Methionine	4.71
8	Arginine	14.67
9	Phenylalanine	2.96

Table 3: Non-essential amino acids content of *Ceratonia silique L.* pods.

N0	Amino acid	Amount (µg/ml)
1	Aspartic acid	39.86
2	Serine	0.63
3	Proline	232.38
4	Glutamic acid	3.962
5	Glycine	16.07
6	Tyrosine	3.28
7	Alanine	38.85
8	Cystine	9.67

Antimicrobial analysis

The antimicrobial pattern in this study showed that ethanolic extract of *Ceratonia silique L.* pods have the highest zones of inhibition and confirm hypersensitivity to all types of organism included in this study (*Klebsiella pneumonia, Staphylococcus aureus, Escherichia coli, Pseudomonas, Bacillus subtilis* and *Streptococcus*) when using 100% concentration of the extract. Also, there were active inhibition (≥ 10 mm) using 80% concentration for all types of organisms except *Escherichia coli, Pseudomonas,* and *Bacillus subtilis*.

Table 4: Antimicrobial pattern of *Ceratonia silique L.* pods extract on some bacteria.

Concentration (w/v)	Organism					
	Klebsiella pneumonia **(mm)**	***Staphylococcus aureus*** **(mm)**	***Escherichia coli*** **(mm)**	***Pseudomonas*** **(mm)**	***Bacillus subtilis*** **(mm)**	***Streptococcus*** **(mm)**
100%	16	11	17	12	12	12
80%	10	10	0	0	0	12
60%	0	0	0	0	0	0
40%	0	0	0	0	0	0

(%) = Percentage, **(mm)** = millimeter zone of inhibition

DISCUSSION

Plants are of great interest in drug discovery and are the main source of our modern medicine. About 25% of modern medicines are derived from a plant origin and only 5–15% of plants are being investigated for their medicinal use (Khan *et al*., 2019). Bacteria develop resistance against antibiotics due to the presence of multi-drug resistance (MDR) pumps which are predominant in *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, etc. Therefore, researchers are trying to find MDR inhibitors, especially those derived from medicinal plants, due to their diversity to increase the efficacy of antibiotics (Sibanda and Okoh, 2007).

In the present study, the plant under investigation contained different concentrations of flavonoids, terpenoids, tannin, steroids, and saponins. Our results are in the same line with the findings obtained by Luísa *et al*. (2011) who indicated that Cs pods extract has been found to contain alkaloids, tannins, flavonoids, amino acids, in addition to antioxidant and cytotoxic activities. The results of the present study are also in agreement with that reported by Rababah *et al*. (2011) who stated that phenolic compounds are a group of substances such

as phenolic acid, alkaloids, tannins, and flavonoids. Tannins are the most famous ones in this group and contribute to their stypsis.

On the same line, (El Bouzdoudi *et al*., 2016) discussed that carob pods powders from wild trees are richer in polyphenols than those from domesticated trees. Also, pods from trees in the same region showed variable contents in polyphenols, flavonoids, and condensed tannins. Primikyri *et al*. (2014) mentioned that carob is rich in phytochemical compounds that have been shown in the literature to have antitumor, anti-proliferative, and pro-apoptotic activity. These groups of bioactive compounds have been linked with the health-promoting effects of carob in different therapeutic areas, including anti-cancer, anti-diabetes, anti-diarrheal, and anti-hyperlipidemia (Carvalho *et al*., 2016).

Various medicinal plant species are used as food along with their medicinal benefits because of their significant nutritional values (Pandey *et al*., 2006). The amino acid content of carob pods reported in this study agrees with values reported by other investigators (Avallone *et al*., 1997; Sigge *et al*., 2011). The amino acid content of carob pods consists of a mixture of 17 residues (aspartic acid, glutamic acid, serine, glycine, histidine, arginine, threonine, alanine, tyrosine, valine, proline, methionine, isoleucine, leucine, cysteine, phenylalanine, and lysine) (Sigge *et al*., 2011). In general, carob pods can be considered a good source of amino acids according to World Health Organization (WHO) standards for protein. More specifically, the plant contains all seven essential amino acids (threonine, methionine, valine, isoleucine, leucine, phenylalanine, and lysine) at concentrations that meet the WHO standards (Ayaz *et al*., 2009).

Due to their strong effect on microorganisms and antioxidant activities, diets that are rich in phenolic and alkaloid compounds are determined as food with high biological and nutritional values (Ríos and Recio, 2005). Our results in this study are in the same line with the findings reported by Tolentino (1950) who mentioned that *Staphylococcus aureus* is very sensitive to carob pulp substances. In addition, carob can destroy many toxins produced by other types of bacteria such as some strains of *Escherichia coli* and *Vibrio cholerae*. This effect may be due to the presence of phenolic compounds such as tannins. Ben Hsouna *et al*. (1986) indicated that carob pods have a high content of alkaloids and phenolic compounds, which explain the antimicrobial and antifungal activity of the plant. On the other hand, the ethanolic extract of the plant under study, at a concentration of 60 and 40 % did not affect the growth of bacterial strains. It is suggested that a high extract concentration of Cs may contain substances that can inhibit the growth of some microorganisms.

CONCLUSION

Carobs can be considered as a good source of amino acids. The antimicrobial properties of the carob pods make them a potentially interesting ingredient for functional foods. Carob fruits are suggested as excellent herbal supplements and drugs of plant origin.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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