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Effect of Caper *(Capparis spinosa)* Extracts as a Natural Antimicrobial Agent

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ABSTRACT

Since thousands years medicinal plants had been the main natural source of folk and modern drugs. *Capparis spinosa* is one of strongest resistant wild plant to adverse conditions in the Mediterranean area. It has a noticable medicinal and nutritional value. This study aims to determine the antimicrobial activity of alcoholic extracts of flower buds, fruits and leaves of *Capparis spinosa* against a variety of microorganisms causing food poisoning and spoilage [(bacterial strains including *Bacillus subtilis ATCC 14085, Bacillus cereus DSMZ 345, Escherichia coli O157:H7 ATCC 51659, Salmonella typhimurium ATCC 14028, and Staphylococcus aureus ATCC 6528),* (yeast strains including *Candida albicans ATCC 10231 and Geotricum candidum NRRL Y-552)* and (fungal strains including *Aspergillus niger ATCC 102, Aspergillus flavus ATCC 247* and *Fusarium moniliform ATCC 206)*]. Also, the possibility of using caper *(Capparis spinosa)* extracts as natural preservatives in foods production (tomato ketchup) is another objective. The obtained results showed that the strongest antimicrobial activity of both caper buds, fruits and leaves extracts were recorded against *St. aureus* ATCC 6528 and *B. cereus* DSMZ 345 for all studied concentrations, (with diameter of inhibition zone ranged from 16 to 26 mm and, 16 to 25mm, respectively) while the lowest antimicrobial effect was recorded against *G. candidum NRRL Y-552* t and *S. typhimurium* ATCC 14028, with diameter of inhibition zone ranged from 0 to 15 mm and, 12 to 17 mm, respectively. On the other hand, the maximum antifungal effect of all studied extracts was recorded against *F. moniliform* ATCC 206, while the minimum antifungal effect was recorded against *A. niger* ATCC 102. In the same time, sensory properties (texture, taste, color, flavor and Over all acceptability) and total plate count of produced ketchup by adding different concentrations of the previous extracts were evaluated, and the results suggested that, both caper buds, fruits and leaves extracts could be applicable in food processing as natural preservatives.

Keywords: Caper; Antimicrobial activity; Ketchup; Natural preservatives

INTRODUCTION

Newadays, using natural preservatives in Food Processing became more attainable. Natural preservatives of different sources such as microflora, animals and plants were used in food manufacture (Holley and Patel, 2005; Tiwari *et al*., become 2009). Microorganisms control in Foods Processing using Natural antimicrobial agents has become more popular. Fighting harmoful pathogens and spoilage organisms in foods is the main roal of antimicrobial agents, leading that to extend shelf life (Tajkarimi *et al*., 2010).

Regarding to health care in developing countries, Medicinal plants could be considered one of the most impotant factor has a result of its cheapness, efficiency and no side effects (Ashis, 2003).

Using of capper plants for medicinal purposes has been occurred by ancient Romans and Greeks long time ago. Capers could be used as flatulence reducer, anti-rheumatic, liver function improver hepatic protectors and stimulants liver function improver.Also Capers are effective as diuretics, vermifuges arteriosclerosis, kidney disinfectants and tonics. While, The traditional use of caper root boiled extract is treating of gout, dropsy, arthritis and anemia . (Behnaz *et al*., 2012).

Capparis Spinosa is the most important species in *Capparidaceae* family where the genus *Capparis* which had the common name of "Caper". *Capparis spinosa* is one of strongest resistant wild plant to adverse conditions in the Mediterranean region. (Levizon *et al.*, 2004).

Mediterranean basin (Asia, Africa, Europe) and Australia are the main regions in which *Capparis spinosa* is widely spread in winter. Capers is common name of young buds which extremely favored as food spices .At the same time, cosmetics and medicines Processing could utilize the different plant parts (Sozzi, 2001; Rivera *et al*., 2003) .

Caper Leaves and flowers could considered as nature source of flavonoids and polyphenols whereas the poorest part of caper plant is roots. (Lekhmici *et al*., 2012).

The genus of Capparis is known by six species in Egypt (Täckholm, 1974). In Egyptian deserts, *Capparis spinos*a var. aegyptia (Lam.) is a perennial plant growing wildly. Caper fruit and bud are the common parts for edible uses. (Saad and Said, 2011).

For many years, *Capparis sinaica Veill* has been used in the Egyptian folk medicine for many different uses. The presence of bioactive components such as flavonoids, glycosides, sterols, tannins, alkaloids, saponins, resins and carbohydrates has been revealed using Phytochemical screening of the caper (Ghazal *et al*, 2015) .

This investigation aimed to determine the antimicrobial activity of flower buds, fruits and leaves extracts as well as the possibility of their usage as natural preservative in ketchup.

MATERIALS AND METHODS

1. Materials

- Buds, fruits and leaves of Caper (*Capparis spinosa*) plant were collected in 2016, from Wadi El Maghara, North Sinai, Egypt.
- Fresh tomatoes, onion, garlic, vinegar, sugar and salt for preparation of tomato- ketchup were purchased from local market (El- Mataria, Cairo).
- Solvents and all chemicals*:* were obtained from El-Gomhoria Co. Cairo, Egypt.

2. Tested microorganisms

The tested microorganisms strains were brought from Cairo Mircen, Agriculture Faculty-Ain Shams University. Pathogenic and food spoilage microorganisms were used in antimicrobial activity studies, as bacterial strains (*Bacillus subtilis ATCC 14085, Bacillus cereus* DSMZ 345, *Escherichia coli O157:H7 ATCC 51659, Salmonella typhimurium ATCC 14028, and Staphylococcus aureus ATCC 6528),* yeast strains *(Candida albicans ATCC 10231* and *Geotricum candidum NRRL Y-552*), and fungal strains *(Aspergillus niger ATCC 102, Aspergillus flavus ATCC 247* and *Fusarium moniliform ATCC 206).*

3. Preparation of stock extracts

Buds, fruits and leaves were dried on open air and then ground into powder, by Lab. mixer (Monlinex 530, 240V) and were kept for further use in polyethylene bags.

Eight grams of the dried buds, fruits and leaves of Caper (*Capparis spinosa*) were separately submerged in 100 ml of ethanol (8% w/v) and extracted after holding on stand for 72 h. , then filtered using Whatman filter paper No.1 according to (Mann *et al*., 2008).

4. Analytical methods

Phytochemical screening of caper extracts

Phytochemical screening of the caper ethanolic extracts was achieved due to the methods reported by Trease and Evans (1989) for active components detection such as alkaloids, tannins, glycosides, saponins, and phlobatanins. Detection of these phytochemicals was based on visual inspection to either color change or precipitat formation as the result of specific reagents addition as follows:

Alkaloids

3 ml of each caper extract and 1 ml of 1%HCl were added in a test tube, Then heated for 20 min, cooled and filtered. 2 drops of Mayer's reagents were added to 1 ml of the extract. The presence of alkaloids could be indicated by the formation of creamy precipitate.

Tannins

1 ml of 10%KOH (freshly prepared) was added to 1ml of each caper extract. the formation of dirty white precipitate indicated the tannins presence .

Glycosides

10 ml of H2SO4 (50%) was added to 1ml of each caper extract then the mixture heated for15 min in boiling water. The previous mixture with Fehling's solution (10 ml) was boiled.The presence of glycosides could be recognized by the formation of brick – red precipitate.

Saponins

Frothing test: 2 ml of each vigorously shaken caper extract for 2 min in the test tube. No frothing was noticed. (ii) Emulsion test:to 3 ml of each caper extract, 5 drops of olive oil was added to in a test tube then extremely shaken. Stable emulsion formation indicated The presence of saponins.

Flavonoids

1 ml of NaOH (10%) was added to 3 ml each caper extract. yellow coloration is an indicator to the The presence of flavonoids.

Steroids

Salkowski test: concentrated H2SO4 (5 drops) was added to 1ml of each caper extract in test tube. No red colouration indicated the absence of steroids.

Phlobatanins

1ml of each caper extract was added to HCl (1%). Red precipitate formatiom means positive result.

Triterpenes

1ml of each caper extract was added to anhydride acetic acid (5 drops) and concentrated H2SO4 (one drop) was added, then The previous mixture was steamed for 1 h , after that NaOH was used in mixture neutralization then chloroform was added . presence of blue - green color indicates the presence of triterpenes.

Polyphenols determmination

Polyphenols content was determined as total polyphenols according to Swain and Hillis (1959), and all values were expressed as average (mg of Gallic Acid Equivalents/ g of dry base).

HPLC analysis

Phenolic compounds were determined by HPLC according to Biswas *et al*., (2013). Phenolic compounds were Identified by comparing their UV absorption spectrum and retention's time with those of the standards.

5. Assay of antimicrobial activity against bacteria and yeasts

The antimicrobial activity of buds, fruits and leaves of Caper (*Capparis spinosa*) extracts was determined using Paper disc diffusion screening method against bacterial and yeast strains, according to (Li *et al*., 2010) with some modifications as follows: About 3 ml of soft nutrient agar (0.75% agar) containing 1.0% inoculums of bacterial strain was layered over 20 ml of hard nutrient agar containing (2.0 % agar), while about 3 ml of soft potato dextrose agar (0.75 % agar) containing 1.0% inoculums yeast strain was layered over 20 ml of hard potato dextrose agar containing (2.0 % agar). Sterilized filter paper discs (8 mm) were soaked with 30 µl of different concentrations (160, 320, 480, 640 and 800 mg from stock extracts) of prepared buds, fruits and leaves of Caper (*Capparis spinosa*) extracts.

The soaked discs were put in the middle of plates which were contained 1.0% bacterial or yeasts inoculums and the plates were incubated at 37 or 30 ºC for 24 h for bacteria and at 30 °C for 48 h for yeasts. The diameter of the inhibition zone around each of the discs (disc diameter included) was taken as a measure of the antimicrobial activity. All tests were performed in triplicates. Negative control was prepared using ethanol 95% as solvent of buds, fruits and leaves of Caper (*Capparis spinosa*).

6. Determination of the minimal inhibitory concentrations (MICs)

The antimicrobial activity of buds, fruits and leaves of Caper *(Capparis spinosa)* extracts against both Gram-positive, Gram-negative bacteria and yeasts strains were examined by detecting the Minimum Inhibitory Concentrations (MICs), which defined as "the lowest concentration required for complete inhibition of test organism after incubation time in broth media and resulting in large inhibition zones of visible growth"(Zheng and Zhu, 2003; and Li *et al.*, 2010).

7. Antifungal activity (agar dilution method)

The antifungal activity of buds, fruits and Leaves of Caper *(Capparis spinosa)* extracts were estimated using a growth inhibition assay method (Agar dilution method), described by (Guo *et al.*, 2007). The diameters of the largest and smallest fungal colonies were recorded and the averages were calculated. The antifungal in terms of percentage inhibition of mycelia growth (Antifungal index) was calculated after mycelium of fungi reached the edges of the control plates as follows:

$$\textbf{Antifungal index (\%)} = \frac{\textbf{C} - \textbf{E}}{\textbf{C}} \textbf{ X 100}$$

Where C: is the average diameter of the largest and smallest colonies of control groups.

E: is the average diameter of the largest and smallest colonies of the experimental groups.

If the inhibition ratio as antifungal index (%) was greater than 20%, the test strains would be considered inhibited and the minimal inhibitory concentration (MIC) for that strains were then determined.

8. Microbiological examination of ketchup

Different ketchup samples were prepared for microbiological analysis in accordance with ISO 6887-1

(2003) test method for sample preparation titled: (Microbiology of food and animal feeding stuffs - Preparation of test samples, initial suspension and decimal dilutions for microbiological examination). Different samples of 10 g were weighed out from the sterile stomacher zipped bag. Maximum recovery solution (MRD), of 90 ml was added. The sample and MRD solution were blended at low speed for 30 to 60 seconds in stomached machine. A dilution series was prepared by transferring 1ml of the previous dilution to 9ml of (MRD) solution. Different samples were examined for total aerobic bacterial count (ISO 4833-2003).

All previous tests were used to reflect the microbiological quality of the prepared samples. The dilutions were plated onto duplicate plates. Plates were incubated at 30 °C for 2 days for total aerobic bacterial count. Results were expressed as (Cfu /gm of ketchup).

9. Preparation of ketchup

ketchup has been selected as a product to investigate the effect of adding suitable different concentrations (160, 240 and 320 mg /100 ml) of the flower buds, fruits and leaves extracts of caper on microbial quality of the produced ketchup in comparison with positive and negative controls (with and without sodium sorbet 0.1%). ketchup was prepared in the laboratory according to Kumar *et al*. (2015) with slight modification as follows:

Ripe tomato fruits were washed in fresh water to remove dust and dirt particles. Tomatoes were cut and pulped by using a blender and strained through stainless steel strainer to separate seeds and peels. The prepared strained tomatoes were cooked with onion, garlic, sugar, salt and vinegar in a saucepan and brought the mixture to boil and stirring frequently, until volume is reduced by one-half, covered and turned off heat, then, different concentrations (160, 240 and 320 mg /100ml) of the studied caper extracts (flower buds, fruits and leaves) were added. Finally, ketchup was poured into glass jars and stored until evaluation.

10. Sensory evaluation of ketchup

Sensory evaluation of ketchup samples prepared by adding different concentrations of buds, fruits and leaves of caper extracts was determined according to Alam *et al*. (2009). Using 10 panelists from the staff of Agriculture Industrial Unit, Desert Research Center, Mataria, Cairo. Panelists were asked to evaluate texture, taste, color, flavor and over all acceptability.

11. Experimental design

antimicrobial activity of capper organs were acheived in three independent experiments (completely randomized experimental design) .The obtaind results (as mean of triplicate) were evaluated via descriptive analysis (mean and standard deviation) according to Montgomery, D.C. (1997) .

12. Statistical analysis

Sensory evaluation were performed in triplicate and the results are reported as average. Duncan's multiple range test was used to calculate the Significant differences ($p<0.05$) according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

1. Phytochemicals screening

Health beneficial effects of vegetables and fruits could be attributed to their Phytochemical contents (Dahanukar *et al*., 2000). The medicinal properties valu of variable plants could be reflected by the presence of several constituents i.e. terpenoids sesquiterpenes lactones, saponins,, alkaloids, , glycol alkaloids, flavonoids, tannins and alkenyl phenols (Cox, 1990) .Some of them acted as enhance the bioactivity and synergistic of other compounds. Flavonoids and phenols are the most bioactive natural products in medicinal plants (Ngameni *et al*., 2013; Nabavi *et al*., 2015).

Photochemical screening including, polyphenols, flavonoids, steroids, saponins, tannins, glycosides, alkaloids, triterpenes, and phlobatannins of ethanolic extracts of Caper flower buds, fruits and leaves *(Capparis spinosa)* was achieved and the results were tabulated in Table (1)

Table1. Phytochemical screening of ethanolic extracts of Caper buds, fruits and leaves

	Organic Compounds	ethanolic extract of Caper buds flower	ethanolic extract of Caper fruits	ethanolic extract of Caper leaves
1	Polyphenols	+ +	+	+ + +
2	Flavonoids	+ +	+	+ ++
3	Steroids	-	-	-
4	Saponins	-	-	-
5	Tannins	+	+	+
6	Glycosides	+	+	+
7	Alkaloids	+	+	+
8	Triterpenes	-	-	-
9	Phlobatannins	-	-	-

(-) = Absent; (+) = Low concentration, (+ +) = Moderate concentration; and (+ + +) = High concentration

The phytochemical screening illustrated the presence of polyphenols, flavonoids, tannins glycosides and alkaloids with the absence of steroids, saponins, triterpenes and phlobatannins.

These results were similar with findings of earlier studies, supporting *Capparis* enrichment with diverse phytochemical compounds of alkaloids, flavonoids, phenols, and polyphenols (Sharaf *et al*., 2000; Inocencio *et al*.,2000; Tesoriere *et al*., 2007; Mishra *et al*., 2007 and Tlili *et al*., 2010) . Also, Aljaiyash *et al*. (2014) showed that, Presence of alkaloids and or/ nitrogenous bases was only seen in *Capparis spinosea* with the absence of cardiac glycosides, saponins and anthraquinones.

Most of these constitutents have been revealed to possess antimicrobial activity and could be the cause of their recorded activities against some different microorganisms. These results were agreed with (Mahboubi *et al*., 2012) who reported that, caper plant is a rich source of the phenolic compounds, vitamin C, carotenoids, tocopherols, and rutin which could be responsible for its antimicrobial effects.

2. Polyphenols content

Secondary plant metabolites include a large and diverse group known as polyphenols, so they are widely found in most fruits,vegetables and herbs, even in higher amounts than vitamins. Polyphenols possess many biological effects, therfore they thought to have potential important human nutrients. Mojzer *et al*. (2016) focused their efforts on the polyphenols bioavailability, anticarcinogenic and antioxidative properties. Phenolic acids and flavonoids are The most commonly dietary polyphenols. (Araújo *et al*., 2011). Flavonoids characterized anti-thrombotic, antibacterial, anti-allergic and anti-inflammatory actions (Biesaga , 2011).

The total polyphenol contents of Caper buds, fruits and leaves extracts were analysed and the obtained data were illustrated in Table (2).

Table 2. Total polyphenol contents of Caper buds, fruits and leaves.

Sample	Total polyphenols (mg gallic / g)
Flower buds	38.332± 0.232
Fruits	34.813± 0.013
Leaves	39.965± 0.014

The results obtained in Table (2) showed that, the highest phenolic content was found in leaves extract (39.965 mg gallic acid/g sample) followed by buds extract (38.332 mg/g) and the lowest was in fruits extract (34.813 mg/g). These results were similar with those of (Tlili *et al*., 2010) who showed that *Capparis spinosa* leaves contain higher level of phenolic compounds than buds.

Also, Mahboubi and Mahboubi (2014) reported that total polyphenols content of fruit *(C. spinosa)* methanol and ethanol extracts were ranged from 31.7 to 34.2 mg GAC/g respectively, and ethanolic caper fruit extract only possessed higher activity against both of Gram-negative bacteria *and Streptococcus* sp. Therfor, roots and fruits of *Capparis spinosa* could be useful in treatment of bacterial infection either for diarrhea and hemorrhoids, as its conventional uses.

3. HPLC evaluation

Identification of the phenolic compounds of Caper buds, fruits and leaves extracts were carried out using HPLC and the results were listed in Table (3).

Table 3. Content of quercetin, kaempferol and the phenolic compounds of Caper buds, fruits and leaves extracts using HPLC analysis.

Compound	Area %		
	buds	Fruits	Leaves
Quercetin	39.37	37.98	87.47
kaempherol	42.70	35.65	11.45
ferulic acid	n.d*	n.d*	n.d*
Resorcinol	n.d*	0.02	0.12
Naphthaline	n.d*	0.97	0.02

n.d*: Not detected

The results obtained in Table (3) indicated that two main flavonoids were detected in all the analysed samples. These identified flavonoids were quercetin and kaempferol. The highest amount of quercetin was observed in the leaves of caper extract (87.47g/100g) comparing with the buds and fruits extracts (39.37 and 37.98 g/100g).While, the second major identified flavonoid was Kaempferol which had the highest content in Caper buds extract (42.70 g/100g), followed by fruits (35.65 g/100g), while the lowest amount was in caper leaves extract (11.45 g/100g). On the other side, at the same table it could be noticed that resorcinol and naphthaline were detected in fruits and Leaves in little quantities, whereas they not detected in buds (Lekhmici *et al*., 2012). Roots of *Capparis spinosa* were poor in either flavonoids or polyphenols while Leaves and flowers were rich. Quercetin was quantitatively determined in different plant parts of C. spinosa at the mature fruiting stage. Quercetin content varied from 1.7 mg/g to 12.8 mg/g among different parts of caper. Flower, floral bud and fruit had higher content of quercetin respectively (Behnaz *et al*., 2012). Flavonoids are found in leafy vegetables and fruits (a big family of polyphenolic compounds had been formed from the base substance flavone by plants). Flavonoids thought to enhance potential and multifarious health benefits accourding to chelating activity and radical scavenging. (Tapan Seal, 2016).

4. Antimicrobial activity

The antimicrobial activity of Caper buds extract against some selected micro organisms was evaluated by inhibition zones absence or presence and the results were showed in Table (4). The obtained data in Table (4) illustrated obviously that, the higher the concentrations of Caper buds extract the higher the antimicrobial activity, but the strongest antimicrobial activity was recorded against *St. aureus* ATCC 6528 and *B. cereus* DSMZ 345 for all studied concentrations, (with diameter of inhibition zone ranged from 16 to 26 mm and, 16 to 25mm,respectively) while the lowest antimicrobial effect was recorded against *G. candidum NRRL Y-552* t and *S. typhimurium* ATCC 14028, (with diameter of inhibition zone ranged from 0 to 15 mm and, 12 to 17 mm, respectively) .

Table 4. Antimicrobial activity of Caper buds extract at different concentrations (mg/100ml).

Bacterial strains	Concentration (mg/100ml)					MIC** mg/100ml
	160	320	480	640	800	
	*Diameter of Inhibition Zones (mm.)					
B. subtilis ATCC 14085	14 ± 1	16 ± 1	20 ± 2	20 ± 1	21 ± 2	480
B. cereus DSMZ 345	16 ± 0	19 ± 1	23 ± 2	25 ± 1	25 ± 2	640
E. coli O157:H7 ATCC 51659	14 ± 1	16 ± 1	19 ± 2	19 ± 2	19 ± 1	480
S. typhimurium ATCC 14028	12 ± 0	13 ± 1	15 ± 1	17 ± 2	17 ± 1	640
St. aureus ATCC 6528	16 ± 1	17 ± 2	20 ± 2	26 ± 2	26 ± 1	640
C. albicans ATCC 10231	0 ± 0	14 ± 0	17 ± 1	24 ± 2	24 ± 1	640
G. candidum NRRL Y-552	0 ± 0	10 ± 1	13 ± 0	15 ± 1	15 ± 1	640

*** Including disc diameter of (8 mm). ** MIC: Minimal inhibitory concentrations**

Data in the same Table also showed that, minimum inhibitory concentration (MIC) of Caper buds extract was 480 mg /100ml against *B. subtilis* ATCC 14085and *E. coli* O157:H7 ATCC 51659 while, it was 640 mg/100ml of the other studied organisms.

Data in Table (5) showed that the antifungal activity of ethanolic Caper buds extract at different concentrations against selected fungal strains. The presented results showed that, the minimum inhibitory concentration (MIC) were 640 mg/100 ml for *A. flavus* ATCC 247 and 480 mg /100ml *F. moniliform* ATCC 206 , while *A. niger* ATCC 102 had noticeable resistance for all studied concentrations.

From the same Table it could be noticed that all fungal strains (*A. niger ATCC 102, A. flavus ATCC 247* and *F. moniliform ATCC 206)* were not effective at the lowest concentration (160 mg /100ml) of Caper buds extract while, the strongest antifungal activity was recorded against *F. moniliform* ATCC 206 (Antifungal index ranging from 12 to 48%).

The presented data in Table (6) showed that the antimicrobial activity of ethanolic Caper fruits extract

against some selected micro organisms, where the minimum inhibitory concentration (MIC) were 480 mg/100ml (w/v) against *B. cereus DSMZ 345, C. albicans ATCC 10231and G. candidum NRRL Y-552* ,while it was 640 mg/100 ml MIC for the other studied microorganisms.

Table 5. Antifungal activity of Caper buds extract at different concentrations (mg/100ml).

Bacterial strains	Concentration (mg/100ml)					MIC** mg/100ml
	160	320	480	640	800	
	Antifungal index (%)					
A. niger ATCC 102	0 ± 0	0 ± 0	0 ± 0	13 ± 1	16 ± 0.5	---
A. flavus ATCC 247	0 ± 0	0 ± 0	17 ± 0.6	25±1.5	31± 0.7	640
F. moniliform ATCC 206	0 ± 0	12 ± 0.5	27 ± 1.5	44 ± 1	48 ± 0.6	480

Table 6. Antimicrobial activity of Caper fruits extract at different concentrations (mg/100ml).

Bacterial strains	Concentration (mg/100ml)					MIC** mg/100ml
	160	320	480	640	800	
	*Diameter of Inhibition Zones (mm.)					
B. subtilis ATCC 14085	14 ± 1	19 ± 1	23 ± 0	25 ± 1	25 ± 1	640
B. cereus DSMZ 345	17 ± 1	21 ± 1	26 ± 2	26 ± 1	27 ± 2	480
E. coli O157:H7 ATCC 51659	12 ± 0	15 ± 1	17 ± 2	20 ± 1	20 ± 2	640
S. typhimurium ATCC 14028	11 ± 1	14 ± 1	15 ± 1	18 ± 0	18 ± 1	640
St. aureus ATCC 6528	14 ± 1	19 ± 1	24 ± 1	25 ± 0	25 ± 1	640
C. albicans ATCC 10231	12 ± 1	17 ± 1	22 ± 1	22 ± 0	22 ± 1	480
G. candidum NRRL Y-552	10 ± 0	13 ± 1	16 ± 1	16 ± 0	17 ± 1	480

*** Including disc diameter of (8 mm). ** MIC: Minimal inhibitory concentrations**

Data in the same Table demonstrated that, the highest antimicrobial activity was recorded against *B. cereus DSMZ 345* , with diameter of inhibition zone ranged from 17 to 27 mm, followed by both *B. subtilis* ATCC 14085 and *St. aureus* ATCC 6528 with diameter of inhibition zone ranged from 14 to 25 mm. while , the lowest antimicrobial effect was recorded against *G. candidum NRRL Y-552 typhimurium* ATCC 14028 *and E. coli, S.* O157:H7 ATCC 51659 with diameter of inhibition zone ranged from 10 to 17 mm, 11 to 18 mm and 12 to 20 mm, respectively.

These results were in acceptance with Fatima-Zahra *et al*. (2017) who concluded that, the ethanolic extracts of fruits and flower buds of *C.spinosa* have more interesting antibacterial activities in vitro than aqueous extracts on multi-resistant pathogenic germs belonging to the Gram-positive and negative. Also, Mahboubi and Mahboubi (2014) studied the antimicrobial activity of methanol, ethanol, ethyl acetate, and aqueous extracts for both roots and fruits of *Capparis spinosa* (separately) against a various of micro organisms and they found that ethanolic Caper fruits extract had the most activity against Gram-negative bacteria and *Streaptococcus* sp.

The antifungal activity of ethanolic extract of Caper fruits was studied against selected fungal strains (*A. niger* ATCC 102 , *A. flavus* ATCC 247 *and F. moniliform* ATCC 206) and the obtained results were listed in Table (7) .

Table 7. Antifungal activity of Caper fruits extract at different concentrations (mg/100ml).

Bacterial strains	Concentration (mg/100ml)					MIC** mg/100ml
	160	320	480	640	800	
	Antifungal index (%)					
A. niger ATCC 102	0 ± 0	8 ± 0.3	14 ± 0.5	22 ±1	31 ± 0.8	640
A. flavus ATCC 247	0 ± 0	15 ± 0.5	23 ±1.2	29 ±1	37 ± 0.8	480
F. moniliform ATCC 206	14±0.5	18 ± 1	28 ± 0	37 ±0.6	42 ±1.2	480

The results illustrated that the concentration of 160 mg/100ml of caper fruits was not affected against *A. niger* ATCC 102 *and A. flavus* ATCC 247, but using the higher concentrations of Caper fruits extract over (160 mg/100ml) led to increase Antifungal index , and the highest value was found against *F. moniliform* ATCC 206 (ranged from 14 to 42 %) where the minimum inhibitory concentration (MIC) was 480 mg/100 ml. while the lowest antifungal effect was recorded against *A. niger* ATCC 102 with antifungal index ranged from 0 to 31 % and the minimum inhibitory concentration (MIC) was 640 mg/100 ml .

The presented data in Table (8) demonstrated that the ethanolic extract which was prepared from Caper leaves generally has inhibitory effects against all tested bacterial strains. It is clear that the strongest antimicrobial activity was recorded against *B. cereus* DSMZ 345 with diameter of inhibition zone ranged from15 to 24 mm, while the lowest antimicrobial effect was found against *S. typhimurium* ATCC 14028 with diameter of inhibition zone ranged from 11-15mm, and a moderate activity (19 mm) was recorded for *both E. coli* O157:H7 ATC 51659 and *G. candidum NRRL Y-552*.

Table 8. Antimicrobial activity of Caper leaves extract at different concentrations (mg/100ml).

Bacterial strains	Concentration (mg/100ml)					MIC** mg/100ml
	160	320	480	640	800	
	*Diameter of Inhibition Zones (mm.)					
B. subtilis ATCC 14085	13 ± 1	15 ± 1	19 ± 2	19 ± 1	21 ± 0	480
B. cereus DSMZ 345	15 ± 1	18 ± 1	24 ± 2	24 ± 1	24 ± 1	480
E. coli O157:H7 ATCC 51659	13 ± 1	15 ± 1	18 ± 2	18 ± 1	19 ± 1	480
S. typhimurium ATCC 14028	11 ± 1	12 ± 0	15 ± 1	15 ± 2	15 ± 1	480
St. aureus ATCC 6528	15 ± 0	17 ± 1	23 ± 1	22 ± 1	24 ± 1	480
C. albicans ATCC 10231	11 ± 0	14 ± 1	19 ±1	23 ± 2	23 ± 0	640
G. candidum NRRL Y-552	10 ± 1	12± 0	17±1	17 ± 1	19 ±1	480

*** Including disc diameter of (8 mm). ** MIC: Minimal inhibitory concentrations.**

Results in the same Table also reported that, minimum inhibitory concentration (MIC) of leaves extract was 640 (mg /100ml) against *C. albicans ATCC 10231*and 480 (mg/100ml) for all the other studied microbial strains.

Benakashani *et al*. (2016) studied the antimicrobial effect of silver nano-particles which were produced using the extract of *Capparis spinosa* leaves (NPs) against some selected pathogenic bacteria strains such as *Salmonella typhimurium, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli*, They found that, the synthesized silver nanoparticles in *Capparis spinosa* leaves extract, showed an excellent antibacterial property.

Data in Table (9) showed that the antifungal activity of ethanolic caper leaves extract at different concentrations against selected fungal strains. The presented results demonstrated that,the minimum inhibitory concentration (MIC) was 800 (mg /100 ml) for *A. niger ATCC102* , and 480 (mg/100 ml) *for A. flavus ATCC 247* and *F. moniliform ATCC 206*.where the most sensitive fungi was *F. moniliform ATCC 206* with Antifungal index ranged between 17 – 41% , followed by *A. flavus ATCC 247* .

These results could be confirmed by different studies such as, Gaind and Juneja (1969) who demonstrated that, different part extracts of *Capparis* species show biological activity against large numbers of pathogens. Also, Sama and Ajaiyeoba (2006) studied the antimicrobial activities of ethanol extracts of aerial part (stem and leaf) of *Capparis thonningi* and *Capparis tomentosa* and cleared that, the ethanol extract of *Capparis tomentosa* inhibited growth of *Staphylococcus aureus, Bacillus cereus, Aspergillus flavus* and *candida albicans.*

Table 9. Antifungal activity of Caper leaves extract at different concentrations (mg/100ml).

Bacterial strains	Concentration (mg/100ml)					MIC** mg/100ml
	160	320	480	640	800	
	Antifungal index (%)					
A. niger ATCC 102	0 ± 0	8 ± 0.5	12±1	19±1.5	25±2	800
A. flavus ATCC 247	0 ± 0	13±0.3	21±0.5	24±0.5	29±1	480
F. moniliform ATCC 206	0 ± 0	17±1	23±0.5	35±1.5	41±0.8	480

Antifungal activity of *C. spinosa* extracts showed that, *R. stolonifer* and *F. moniliforme* were the most sensitive fungi with inhibition zone 25 and 21 mm respectively whereas *A.niger* was the most resistant organism. The aqueous extract from total aerial parts of the plant has been used for its antifungal activity. *Capparis spinosa* could be recognized as an important source for antifungal medicine (Ali-Shtayeh and Abu Ghdeib, 1999).

5. Ketchup Examination

Ketchup samples were produced using different concentrations of both capper buds, fruits and leaves were microbiological and sensorial assessed and the achieved results were presented in Table 10 and 11, respectively.

The presented data in Table (10) showed that the higher the concentration of studied extracts for each source the higher microbial growth inhibition. Where the caper buds extract had the lowest total plate count of produced Ketchup and had been recorded 1.4 X 102 Cfu/g followed by Leaves extract which was 1.8 X 102 Cfu/g at the same concentration (320 mg/100ml) comparing with negative Control (without any preservatives) and positive Control (*0.1 % sodium sorbet*) 8.7 X 103 and 5.1 X 103 Cfu/g respectively.

Organoleptic characteristics are an important index of potential consumer favorability. The sensory quality characteristics of produced Ketchup by adding different concentration of Caper buds, fruits and leaves extracts were presented in Table (11).

In general, control samples (positive and negative) showed the highest total score (46 out of 50 points) among all treatments, it seemed that the manufactured Ketchup with adding fruits extract at 160 mg/100ml, had higher total score (42 out of 50 points) followed by fruits extract at 240 mg/100ml, which recorded 41.7. On the other hand, leaves extract addition at 320 mg /100ml, recorded the lowest total score (33.79 out of 50 point).

Table 10. Total plate count (Cfu/g) produced Ketchup with different concentrations of Caper buds, fruits and Leaves extracts.

Samples	TPC (Cfu/g)
Control (-) *without any preservatives*	$8.7 \times 10^3 \pm 0.05$
Control (+) *0.1 % sodium sorbet*	$5.1 \times 10^3 \pm 0.02$
Buds extract (160 mg /100ml)	$5.3 \times 10^3 \pm 0.03$
Buds extract (240 mg /100ml)	$5.2 \times 10^2 \pm 0.02$
Buds extract (320 mg /100ml)	$1.4 \times 10^2 \pm 0.04$
Fruits extract (160 mg /100ml)	$7.1 \times 10^3 \pm 0.06$
Fruits extract (240 mg /100ml)	$4.9 \times 10^2 \pm 0.03$
Fruits extract (320 mg /100ml)	$2.8 \times 10^2 \pm 0.06$
Leave extract (160 mg/100ml)	$5.8 \times 10^2 \pm 0.08$
Leave extract (240 mg /100ml)	$3.o \times 10^2 \pm 0.06$
Leave extract (320 mg /100ml)	$1.8 \times 10^2 \pm 0.05$

(CFU or cfu): Colony-forming unit TPC: Total plate count

Table 11. Sensory properties of produced Ketchup by adding different concentrations of Caper buds and fruits Leaves extracts.

Samples Extracts Concentrations	Texture (10)	Taste (10)	Color (10)	Flavor (10)	Over all Acceptability (10)	Total Score (50)
Control (-) *without any preservatives*	9.357 a	9.500 a	8.857 c	9.214 a	9.286 a	46.214 a
Control (+) *0.1 % sodium sorbet*	9.500 a	9.214 b	9.286 a	8.929 b	9.357 a	46.286 a
Buds extract (160 mg/100 ml)	8.857 c	7.286 f	9.000 b	7.357 f	7.786 e	40.286 c
Buds extract (240 mg/100 ml)	9.000 bc	6.500h	8.071 e	7.500 e	7.857 e	38.928 d
Buds extract (320 mg/100 ml)	9.071 b	6.071j	7.786 g	7.142 g	7.071h	37.143 e
fruits extract (160mg/100 ml)	9.071 b	7.714 d	9.000 b	8.000 c	8.286 c	42.071 b
fruits extract (240 mg/100 ml)	9.000 bc	7.500 e	8.857 c	7.928 c	8.429 b	41.714 b
fruits extract (320 mg/100 ml)	8.928 bc	7.857 c	8.357 d	7.643 d	8.071 d	40.571 c
Leaves extract (160 mg/100 ml)	8.857 c	7.071 g	7.857 f	7.286 f	7.500 f	38.786 d
Leaves extract (240 mg/100ml)	8.286 e	6. 286 i	7.429 h	6.786 h	7.214 g	36.001 f
Leaves extract (320 mg/100ml)	8.500 d	5.214 k	6.714 i	6.571 i	6.786 i	33.786 g
L.S.D	0.159	0.122	0.066	0.112	0.110	0.401

Values bearing the same letter within the same column are not significantly different (P> 0.05)

Results at the same in Table (11), were also demonstrated that, all studied sensory attributes recorded considerable high levels of acceptance for Caper fruits extract in comparison with both of buds and leaves treatments. Meanwhile, The lowest organoleptic characteristics were possessed for Ketchup produced by adding leaves extract at 320 mg /100ml, especially moderately bitter taste which detected by panelists.

CONCLUSION

From all results which had been discussed in this study, it could be concluded that, the ethanolic flower buds, fruits and leaves extracts of caper *(Capparis spinosa) are* suitable for fighting bacterial pathogens especially positive and negative Gram bacteria, yeasts and Fungi strains, (this could be attributed to the high amount of bioactive compounds which have been found in tested caper extracts). Ketchup examination results (either sensory or microbiology) suggested that all of them could be used as an important source of natural preservatives for food manufacture.

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تأثير مستخلصات اللصف كعامل طبيعى مضاد للميكروبات

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منذ آلاف السنين كانت الطبيعة مصدرا رئيسيا للأدوية وتم فصل العديد من العقاقير الحديثة من مصادر طبيعية . وقد أظهرنبات اللصف (*Capparis spinosa*) مقاومة شديدة للظروف الغير مناسبة فى منطقة البحر المتوسط ' كما أن له قيمة طبية وغذائية . لذلك كان الهدف من هذه الدراسة تقدير النشاط المضاد للميكروبات للمستخلصات الكحولية لكلا من البراعم والثماروالأوراق لنبات اللصف ضد مجموعة من الكائنات الحية الدقيقة المتنوعة المسببة للتسمم وفساد الأغذية {السلالات البكتيرية وتشمل *Bacillus subtilis ATCC 14085 - Escherichia coli O157:H7* ATCC *5165) Staphylococcus aureus ATCC 6528 -- Salmonella typhimurium ATCC 14028* (الفطريات وتشمل *Geotricum candidum NRRL Y-552 - Candida albicans ATCC 10231)* الخمائروتشمل *Bacillus cereus DSMZ 345 - Fusarium moniliform ATCC 206 - Aspergillus flavus ATCC 247 - Aspergillus niger ATCC 102*) . ايضا إمكانية إستخدام هذه المستخلصات كمواد حافظة طبيعية فى إنتاج الأغذية (الكاتشب) يعتبرهدفا أخرللدراسة . وأظهرت النتائج المتحصل عليها أن مستخلصات كلا من براعم وثماروأوراق نبات اللصف كان لها تأثير قوى مضاد للميكروبات ضد كلا من *Staphylococcus aureus* و *Bacillus* وذلك عند التركيزات محل الدراسة (مع قطرمنطقة تثبيط تراوح مابين 16- 26مم , 16- 25مم على التوالى) . بينما سجل أقل تأثير مضاد للميكروبات ضد كلا"من NRRL Y-552 *G. candidum* و *Salmonella typhimurium ATCC 14028* (مع قطرمنطقة تثبيط تراوح مابين صفر- 15مم , 12- 17مم على التوالى) .من جهة اخرى كان أقصى تأثير مضاد للفطريات لكل المستخلصات محل الدراسة سجل ضد الفطر *Fusarium moniliform ATCC 206* بينما سجل أقل تأثير مضاد للفطريات ضد *Aspergillus niger ATCC 102* . فى نفس الوقت أظهرت نتائج التقييم الحسى (القوام – الطعم – اللون – النكهة – القبول العام) والعد الكلى للميكروبات للكاتشب المنتج بواسطة إضافة تركيزات مختلفة من المستخلصات السابقة الذكرأن كلا من مستخلصات البراعم والثماروالأوراق لنبات اللصف يمكن إستخدامها تطبيقيا فى تصنيع الأغذية كمواد حافظة طبيعية .