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*RESEARCH ARTICLE*

# ANTIBACTERIAL SYNERGISTIC EFFECT OF EXTRACTS OF THE ORGANS OF *CAPPARIS SPINOSA* AND IN COMBINATION WITH ANTIBIOTICS.

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## Abstract

**Background:** The objective of this study is to test the antibacterial potency and evaluate the possible synergistic effect between aqueous and ethanolic extracts, flower buds and fruits of *Capparis spinosa*, and between these extracts and antibiotics.
**Materials and Methods:** Antibacterial activity was determined by the plaque microdilution method against seven pathogenic and multi-resistant bacteria. Concerning the synergistic effect, between the aqueous and alcoholic extracts of the two organs of this plant, it was carried out according to the "Checkerboard" method. The synergy between the extracts and the antibiotics was estimated by comparing the diameters of the zones of inhibition on solid medium.
**Results:** The extracts showed interesting antibacterial activity with MICs varying from 1.73 ± 0.04 to 66.66 ± 0.00 mg / ml.
Both combinations carried between the extracts of flower buds and fruits, ethanolic and aqueous, against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* showed a synergistic effect with ICIF ranging from 0.02 to 0.24.
The association between the four extracts and ten antibiotics on the seven strains tested showed an increase in the antibacterial potency of the antibiotic from 11 combinations ranging from 66% to 471%.
**Conclusion:** The present study show that these tested extracts inhibit the growth of Gram-positive and Gram-negative bacteria, which allows us to deduce their broadened spectrum of action and exploit the efficacy of their bioactive molecules as adjuvants to antibiotics.



## Introduction:-

Regarding of the continuing increase in the resistance of bacteria to antibiotics, the emergence of multi-resistant strains and the resulting therapeutic problems, the search for other antimicrobial opportunities is necessary. The use of herbal medicine is one of the promising solutions if it is based on scientific studies. Recently, several data are published in this direction, making it possible to value and rationalize the beneficial effect on health of aromatic and medicinal plants (Schwalbe et al., 2007).

The interest of interactions between active components of the same plant and between two or more plants has attracted the attention of several researchers on a global scale (Rosato et al., 2007). Indeed, since the discovery of

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the synergistic and antagonistic effects and their importance in the therapeutic field, the interaction between the biologically active agents have become an important subject for scientific research to solve the problem of bacterial resistance and to reduce possible side effects of treatments by decreasing the dose of compound used (Rosato et al., 2007). In addition, the use of two to four antibiotics in combination is sometimes necessary to remedy several diseases like skin infections, respiratory, acute exacerbation of chronic obstructive pulmonary disease, urinary tract infections, the intra-abdominal infections, of which the responsible germs are *Staphylococcus aureus* SARM, *Pseudomonas, E. coli, Klebsiella* (Le comité provincial thérapeutique, 2016).

In this context, our contribution in the fight against the emergence of antibiotic-resistant bacteria will focus on the study of the increase in antibacterial efficacy of the *Capparis spinosa* plant, known for its impressive therapeutic properties (Aissani, 2013), making it undergo a varied combination between the extracts of its various organs and or with antibiotics of weakened efficacy by the acquisition of bacterial resistances.

## Material and methods:-

### Plant material:-

*Capparis spinosa* called the Caper is a spontaneous shrubby plant (Satyanarayana et al., 2008). This plant is very widespread in the mediterranean basin. It is also widely cultivated in the dry regions of West and Central Asia (Aissani, 2013). Its flower buds (Capers) and immature fruits are consumed as foods or condiments in the kitchen (Tesoriere et al., 2007). The caper with its different parts, flower buds, fruits, seeds, shoots and bark roots, has several medicinal qualities, which explains its intense use in traditional medicine.

The collection of the plant material was done in June of 2015. It consists of flower buds and mature fruits of the species *Capparis spinosa* harvested from Teghari (Latitude: 34.412086; Longitude: -6.044096), located in the province of Sidi Kacem in Morocco.

The species has been identified in the laboratory of plant biotechnology and molecular biology of the Faculty of Sciences of Moulay Ismail University, Meknes (Ennacerie et al., 2017). This plant material was sorted and then dried in the shade at room temperature. A portion of the dried plant was ground to an electric mill to obtain a fine powder which would be used for the preparation of the extracts.

### Bacterial strains tested:-

To evaluate the antibacterial activity of *C. spinosa* extracts, seven multi-resistant pathogenic bacteria were selected. Six were clinically isolated from various pathological products from a private medical laboratory, and *Listeria monocytogenes* was provided by the CNRST Laboratory of Microbiology and Molecular Biology (LMBM). Their removal and isolation were carried out in accordance with hygiene standards and using the appropriate selective culture media.

These strains were tested for both Gram-positive: *Staphylococcus aureus* sensitive to methicillin (SASM), *Staphylococcus aureus* resistant to methicillin (SARM) and *Listeria monocytogenes.* As or the Gram negative: *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Salmonella sp.*

### Methods of preparing plant extracts:-

Two types of aqueous and ethanolic extract were prepared from powdered of the flower buds and fruits of *C. spinosa*.

### Aqueous extract:-

One hundred grams of the plant powder of each organ of the plant is boiled for 15 minutes in a liter of distilled water. This cooled 10% decoctate was filtered successively on hydrophilic cotton and on Wattman paper n° 1. The filtrate obtained was then placed in an oven at 55 °C until desiccation.

### Ethanolic extract:-

The ethanolic extract is obtained with magnetic stirring at room temperature by successive maceration of 100 g of the plant powder of each organ of the plant in a volume of 300 ml of ethanol for 24 hours. Then, the extract is filtered with hydrophilic cotton and then with Wattman paper n° 1. The collected filtrate is dried in an oven at 50 °C for the removal of ethanol and a dry residue expressed in mg. This makes it possible to express the precise concentrations of dry residues soluble in Dimethylsulfoxide (DMSO) in mg / ml.

**Study of antibacterial activity:-**

**Preparation of inoculum:-**

The inoculum is prepared from a bacterial culture of 18 to 24 hours. It is cultured on agar medium and incubated at 37°C, by taking several colonies of the same morphology and suspending them in a sterile solution of physiological water at concentration 9 g / l of NaCl, to have an inoculum with a density equivalent to the standard 0.5 Mc Farland (Anon, 2015).

**Determination of the minimum inhibitory concentration (MIC):-**

The MIC is the minimum concentration of extract or essential oil that inhibits the growth of 90% of the bacterial population after incubation for 18-24 hours at 37 ° C. The determination of the MICs of the extracts of the plant, with respect to the bacterial strains is carried out according to the microtitration technique on microplates. It consists of the distribution of 50 µl of Muller Hinton broth on all the wells. Then 50 µl of the stock solution of the extract to be tested are added to the first well of each line from which a series of dilutions of geometric reason 2 are made. Each well is then seeded with 50 µl of the bacterial suspension at $5.10^5$UFC/ml (Ellof, 1998), (Chebaibi et al., 2011). The microplates are then incubated at 37 ° C for 18-24 hours. At the end of the incubation period, 2,3,5-triphenyl-2H-tetrazolium chloride (TTC), which is used as a viability indicator, is prepared extemporaneously at 0.4 mg / ml in sterile physiological water (0.9% NaCl). Then 20µl of this solution are added to each well. The plate is then reincubated for 10 to 30 min at 37 ° C. Wells where bacterial growth occurred showed a pink color.

The growth controls are prepared in isolated wells containing the culture medium and the bacterial strains tested, but without addition of the extract studied. The tests should be repeated three times.

**Determination of minimum bactericidal concentration (MBC):-**

The minimum bactericidal concentration (MBC) is the lowest concentration of the extract that leaves at most 0.01% of surviving germs. Using a 2 µl-calibrated loop, the contents of wells in which no growth was observed were harvested and seeded on Mueller-Hinton agar starting from the MIC well (Koné et al., 2004), (Yao et al., 2016). The MBC of the product is deduced from the first petri dish free of bacteria. Each experiment is performed three times in three successive experiments.

Referring to the MBC / MIC report, the antibacterial effect can be judged;
If the MBC / MIC <4 the effect is bactericidal, and if CMB / MIC> 4 the effect is bacteriostatic (Berche and Gaillard, 1991), (Bouharb at al., 2014).

**Synergy test between extracts: "Checkerboard" method**

This method is based first of all on the preparation of the stock solutions and serial dilutions of each extract, starting with a concentration at least equal to twice the MIC, these dilutions are used to prepare the various combinations. In each well of the microplate, 50 µl of Mueller-Hinton broth were introduced and then 50 µl of the first extract A with double MIC was added, in order to be diluted in series along the wells of the ordinate of the first line, while the second extract B of the same volume and concentration is diluted on the abscissae of the first line. The other wells will contain the combination of the two extracts, so that the concentration of extract A will be the same as that of the first well of ordinate on the same line, and that of extract B will be equal to that of the first well of the abscissae on the same line. Subsequently, 100 µl of the bacterial inoculum of $5 \times 10^5$ CFU / ml is added to each well of the plate. Incubation of the plates is carried out at 35 ° C for 24 hours under aerobic conditions (Orhan et al., 2005).
The interpretation of the results is done by calculating the fractional inhibitory concentration index ICFI:

**FICI = ΣFIC = FIC A + FIC B**

Where, **FIC A** is the MIC of extract A in the combination / MIC of extract A alone,
and **CFI B** is the MIC of extract B in the combination / MIC of extract B alone ;
So reading the results will be taken into account the values found of the FICI ;

**- FICI ≤ 0,5: Synergistic effect;**
**- 0,5 <FICI <2: Indifferent effect;**
**- FICI ≥2: Antagonistic effect.**

**Synergy test between extracts and antibiotics: Disc diffusion method:-**

For each strain two antibiotics to which they are resistant are tested. After flooding of the cans with the prepared inoculum. A disc saturated with 20µl of the extract and two disks of the antibiotics are deposited on the surface of the agar. To evaluate the combination effect, 20µl of the extract is added to one of the disks of each antibiotic. The

Petri dishes are then left for one hour at room temperature and then incubated at 37 °C for 18 to 24 hours. After incubation, the inhibition diameter is measured in millimeters with disc included (Toure, 2015).

Concerning the negative test, it is presented by a disc impregnated with 10 µl of distilled water for the aqueous extracts and of 10 µl of DMSO for the ethanolic extracts.
The interaction of the antibiotic and the extract may produce four main types of effects:

1. **Indifference:** the activity of the extract has no influence on the activity of the antibiotic;
2. **Addition:** the effect of the association is equal to the sum of the effects produced by each of the agents taken separately ;
3. **Synergy:** the effect of the association is greater than the sum of the effects produced by each agent taken separately ;
4. **Antagonism:** the effect of the combination is less than the sum of the effects produced by each of the antibiotics taken separately (Ocampo et al., 2014).

## Results and discussion:-

**Extraction:-**

Each extract was characterized by its color and its yield relative to the dry matter. These elements are presented in **Table 1**.

**Table 1**:- Results of yields relative to dry matter, colors and aspect of extracts of flower buds and fruits of *C. spinosa*

| Part studied | Extract | Aspect | Color | Yield (%) |
|---|---|---|---|---|
| **Flower buds** | **Ethanolique** | Viscous | Dark green | 8,380 ± 0,322 |
| | **10% decoctate** | Lyophilised | Brown | 15,500 ±0,02 |
| **Fruits** | **Ethanolique** | Viscous | Dark brown | 7,465 ±0,15 |
| | **10% decoctate** | Lyophilised | Dark brown | 16,810 ± 0,31 |

The yields of the decocts represent double the yields of the alcoholic extracts for the two organs of the plant, flower buds and fruits.

Comparison of these results with those reported by Meddour et al. (2013), shows a difference in the yields of the aqueous and methanolic extracts and which are equal according to them. According to Bouharb et al. (2014), the yield of the aqueous extract was the best, which is in agreement with our results. The difference in yield can be explained by the technique and the parameters of the extraction used, namely the type of solvent, its volume and its polarity, as well as the grain size of the plant material (Bonnaillie et al., 2012).

**Evaluation of the antibacterial potential of extracts:-**

The data on the antibacterial potential of the aqueous and ethanolic extracts prepared from the flower buds and fruits of *C. spinosa* are presented in **Table 2**.

**Table 2:-** Antibacterial Activity: Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC) mg / ml and the MBC / MIC report of extracts of flower buds and fruits of *C. spinosa*.

| Part studied | | | Gram - | | | | Gram + | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *E. coli* | *Klebsiella pneumoniae* | *Salmonelle sp* | *Pseudomonas aeruginosa* | *SASM* | *SARM* | *Listeria monocyto-genes* |
| **Flower buds** | **Ethanolic extract** | **MIC (mg/ml)** | 10,26±0,36 | 13,50±2,58 | 15±1.20 | 12,76±1.16 | 12.5±1.53 | 14,06±1.18 | 13.59±1.38 |
| | | **MBC (mg/ml)** | 41,04±1,47 | 50±4,14 | 60±0.00 | - | 40±2.01 | 36,04±3,54 | 42.08±0.00 |
| | | **MBC/CMI** | 4 | 4 | 4 | - | 3 | 2-3 | 3 |
| | **10% decoctate** | **MIC (mg/ml)** | 66.66±0.00 | - | 50±3.56 | - | 66.66±0.0 | 66.66±0.00 | 23.6±1.53 |
| | | **MBC (mg/ml)** | - | - | - | - | - | - | - |
| | | **MBC/ MIC** | - | - | - | - | - | - | - |
| **Fruits** | **Ethanolic extract** | **MIC (mg/ml)** | 6.25±0.95 | 32.44±3.29 | 21.62±0.2 | 6.25±0.95 | 21.62±1.15 | 8.33±0.93 | 1.73±0.04 |
| | | **MBC (mg/ml)** | 8.33±0.71 | 86.5±4.02 | - | - | 38.29±3.01 | 18.38±2.01 | 8.33±0.66 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | MBC/ MIC | 1-2 | 2-3 | - | - | 2 | 2-3 | >4 |
| | 10% decoctate | MIC (mg/ml) | 41.66±3.36 | 55.55±2.24 | 33.33±0.00 | 33.33±0.00 | 66.66±0.0 | 33.33±0.00 | 38.88±3.57 |
| | | MBC (mg/ml) | 66.66±0.00 | - | - | - | - | - | - |
| | | MBC/ MIC | 2 | - | - | - | - | - | - |

(-): Not determined

***SASM**: Staphylococcus aureus* sensitive to methicillin,
***SARM**: Staphylococcus aureus* resistant to methicillin.

The results show that the various extracts tested have an interesting antibacterial activity in vitro. In general, the two types of the extracts of the flower buds and the fruits have a power of inhibition of the growth of the pathogenic germs of the same efficiency on the Gram-positive and the Gram-negative.

However, by comparing the MICs of the extracts, it appears on the one hand that the aqueous-type extracts of the two organs have relatively similar MICs, ranging from 33.33 ± 0.00 to 66.66± 0.00mg / ml; On the other hand, the MICs of the alcoholic extracts prepared from the flower buds range from 10.26 ± 0.36 to 15 ± 1.20mg / ml and differ from the MICs of the fruit extracts which vary from 1.73 ± 0.04 to 32.44 ± 3.29 mg / ml. This result illustrates that the fruit extract has a more potent power at low concentration compared to the extract of the flower buds. In addition, alcoholic extracts have a higher antibacterial power than decocts for both organs. This finding is similar to that established by Mahasneh Mahasneh (2002), who studied the antibacterial activity of aqueous and ethanolic extracts of caper fruit.

As regards the determination of the MBCs, it was found that the ethanolic extract of the fruit had lower values than those of the flower buds and varied in general between 8.33 ± 0.66 and 86.5 ± 4.02 mg / ml, confirming that the bactericidal potential extracts of the fruit is more intense than that of the flower buds. This power can be explained by the difference in chemical composition of the organs of the same plant and their richness in secondary metabolites such as polyphenols (Lekhmici et al., 2013). As for the aqueous extracts, they have no bactericidal effect against the strains tested except the decoctate of the fruits which has shown its efficacy against the *E. coli* species with a CMB of 66.66 ± 0.00mg / ml.

The calculation of the MBC / MIC report, which informs about the bactericidal effect of the extract, confirms that the ethanolic extract of the fruits generally has a lethal effect against all the germs except *Listeria monocytogenes*. However, it should be pointed out that this extract gave the lowest MIC and MBC compared to the other germs, namely 1.73 and 8.33mg / ml respectively and a MBC / MIC report which is close to 4, thus a rather more pronounced efficacy.

The ethanolic extract prepared from the flower buds generally has a bactericidal effect for all Gram-positive tested and bacteriostatic for all Gram-negative organisms except for *P. aeruginosa* whose effect is indeterminate.

By comparing the tolerance of the seven strains tested with respect to the aforementioned extracts, it is clear that the *P. aeruginosa* species shows inhibited growth without being totally eradicated. However, this result is considerable in view of the innate and acquired resistances of this bacterium. These resistances are due to several factors which can act in parallel such as, the constitutive expression of β-lactamases and / or efflux pumps which do not allow the penetration of antibacterial substances, low permeability of the outer membrane, the adoption of genes coding for antibiotic-destroying enzymes, and the overexpression of efflux pumps, or the reduction of the expression of porins, ... (Mesaros et al., 2007).

Comparing this result with the literature, we note that it is in agreement with that of Mahasneh, (2002) who worked on the aerial part of the plant, as well as that of Meddour et al (2013) who studied its fruits and its flowers.

In other studies, the strain that showed high sensitivity to extracts is *L. monocytogenes*. The same finding was revealed by Proestos et al. (2006) by testing the leaves of *C. spinosa*.

For *Salmonella sp* the ethanolic extracts of the two organs showed a moderate effect compared to that of SARM and SASM, which is in agreement with the results of Proestos et al. (2006).

**Evaluation of the synergistic effect between the extracts:-**
The results of the effect of the combination of aqueous and alcoholic extracts of the two flower buds and fruits of the *C. spinosa* plant are summarized in **Table 3.**

**Table 3**:- effect of the combination of different extracts of the two organs of the *C. spinosa* plant

| Souche testée | FIC of ethanolique extract (FB) | FIC of ethanolique extract (F) | FIC of 10% decoctate (FB) | FIC of 10% decoctate (F) | ICFI | Comparaison | Effect |
|---|---|---|---|---|---|---|---|
| *Klebsiella pneumoniae* | 0.03 | 0.06 | - | - | 0.09 | ≤ 0.5 | Synergy |
| *Pseudomonas aeruginosa* | 0.12 | 0.12 | - | - | 0.24 | ≤ 0.5 | Synergy |
| *Pseudomonas aeruginosa* | - | - | 0.01 | 0.01 | 0.02 | ≤ 0.5 | Synergy |
| *Salmonella sp* | - | - | 0.01 | 0.5 | 0.51 | 0,5<ICIF≤2 | Additive or indifferent association |

**FB**: flower buds ; **F**: Fruits

The Checkerboard microtiter plate synergy test is used to test the activities of the combination of the four extracts against *Klebsiella pneumoniae, Salmonella sp, and P. aeruginosa*, as these bacteria are those that respond less to aqueous extracts and that *P. aeruginosa* is the least sensitive to all extracts. The ICFIs of each combination tested are determined in each case. The combination of the two alcoholic extracts of flower buds and fruits showed ICFIs of 0.024 and 0.09 indicating a very impressive synergistic effect against *K. pneumoniae* and *Pseudomonas aeruginosa.*

Concerning the combination of the two 10% decoctate of the two organs against *Pseudomonas aeruginosa*, it revealed an ICFI of 0.02 a very low value showing a strong synergy. However, the association used against *Salmonella sp* showed a type of interaction classified as an indifferent or additive interaction but tends to be partially synergistic because the ICFI value is 0.51 which is very close to 0.5.

The beneficial effect of the antibacterial capacity acquired by combining the extracts studied can be explained by the fact that the two types of extracts are formed from a set of various phytochemical compounds in which each group can act differently. According to Phillipson and O'Neill (1989), (Olajuyigbe and Afolayan, 2012), the aromatic planar quaternary alkaloids present in the extracts can be intercalated with DNA. Lipophilic flavonoids disrupt the integrity of microbial membranes (Tsuchiya et al., 1996), (Olajuyigbe and Afolayan, 2012), tannins precipitate microbial proteins (Prasad et al., 2008), (Olajuyigbe and Afolayan, 2012), and saponins have detergent properties acting as lytic agents of the cell (Abukakar et al., 2008), (Olajuyigbe and Afolayan, 2012). Because these bioactive plant molecules have a precise bacterial metabolic target, their combination in the extracts will cause the attack of several targets simultaneously, which may make the bacteria more susceptible to their effect, hence the observed synergistic action (Vuuren et al., 2011).

This synergy can also be linked to the interaction of these active ingredients present in the extracts.

**Evaluation of the synergy between extracts and antibiotics on agar medium:-**
The discovery of organic products of antimicrobial capacity, does not allow the replacement of antibiotics. However, these products can be a solution rewarding the high dose of the antibiotics used or also fighting against the resistance acquired by pathogenic germs, and this thanks to the combination between the two.

The results of the combinations tested between the extracts (ethanolic extract of flower buds, 10% decoctate of the flower buds, ethanolic extract of the fruits or 10% decoctate) and ten antibiotics with impaired efficacy in antibiotics on seven bacterial strains are presented in **Table 4.**

**Table 4:-** Effect of association extracts / antibiotic on seven bacterial strains.

| | | Diameters of the inhibition zones (mm) | | | | |
|---|---|---|---|---|---|---|
| | | | Combination of extract and antibiotics | | | |
| **Bacterial strains** | **Sign antibiotic** | **Antibiotic only** | **Ethanolique extract (FB)** | **10% decoctate (FB)** | **Ethanolique extract (F)** | **10% decoctate (F)** |
| **SASM** | **(Extrait seul)** | | *17* | *7* | *12* | *6* |
| | **CN<18** | 13±1.5 | *8* | *<6* | *10* | *<6* |
| | **P** | *24.33±3.77* | *<6* | *<6* | *<6* | *<6* |
| **SARM** | **(Extrait seul)** | | *18* | *6* | *19* | *7* |
| | ***E<18*** | *8*±0.00 | *12* | ***40*** | *8* | *9* |
| | ***CN <18*** | 17.33±0.88 | *13* | *<6* | *11* | *10* |
| ***Listeria monocytogenes*** | **(Extrait seul)** | | *17* | *11* | *25* | *7* |
| | **E<25** | *7±0.00* | ***26*** | ***20*** | ***24*** | ***26*** |
| | **P<13** | 7±0.00 | ***40*** | ***40*** | ***36*** | ***40*** |
| ***Klebsiella pneumoniae*** | **(Extrait seul)** | | *18* | *6* | *8* | *6* |
| | ***CRO<23*** | 16±3.66 | *7* | *<6* | *10* | *<6* |
| | ***C<20*** | 11.5±0.5 | *10* | *10* | *9* | *10* |
| ***Pseudomonas aeruginosa*** | **(Extrait seul)** | | *17* | *6* | *20* | *7* |
| | ***AK<15*** | 14.5±0.44 | *16* | *12* | *16* | *15* |
| | ***IMP<17*** | 12±0.00 | *25* | ***25*** | *30* | ***20*** |
| ***Salmonelle sp*** | **(Extrait seul)** | | *15* | *6* | *12* | *7* |
| | ***CTX<23*** | 10±0.00 | *8* | *12* | *10* | *9* |
| | ***CT<15*** | 13±0.66 | *15* | *15* | *15* | *16* |
| ***Escherichia coli*** | **(Extrait seul)** | | *19* | *6* | *21* | *7* |
| | ***Cip<19*** | 18±0.00 | *14* | *14* | *15* | *17* |
| | ***CN<14*** | 13.33±0.44 | *20* | *14* | *20* | *20* |

***SASM****: Staphylococcus aureus* sensitive to methicillin,
***SARM:*** *Staphylococcus aureus* resistant to methicillin.
**FB**: Flower buds ; **F**: Fruits ; **P**: Pénicilline (10 Unités), **CN**: Gentamicine (10µg), **E**: Erythromycine (15µg), ***CRO:*** Ceftriaxone (30µg), ***C:*** Chloramphénicol (30µg), **IMP**: Imipénème (10µg), **CTX**: Cefotaxime (30µg), **CT**: Colistine (50µg), **Cip**: Ciprofloxacine (5µg), **AK**: Amikacine (30µg)

Among the 56 combinations tested, 11 showed an improvement in the activity of the antibiotic, and 44 had an antagonistic effect whereas a combination showed an indifferent effect. Antibiotics which have shown an improvement in antibacterial potency are Penicillin and Imipenem of the Beta-lactam family and Erythromycin of the family Macrolides.

Improvements in the antibacterial potency of these antibiotics ranged from 66% to 471%, the highest values for antibiotics belonging to the beta-lactam family against *L. monocytogenes*, SARM and *P. aeruginosa* followed by Macrolides.

The general and most probable hypothesis to explain synergism is the destabilization of the bacterial wall. It is possible that the components of the extracts studied allow easier penetration of the molecules of the antibiotics and thus their access to their intracellular target. Precisely, depending on the antibiotic family, this positive interaction can be explained for beta-lactams by inhibiting the molecules involved in bacterial wall synthesis by binding to a Penicillin-Link Protein (PLP). Which inhibits the trans-peptidase property and thus the peptidoglycan synthesis (Tenover et al., 2006). The four extracts showed an acquisition of antibacterial efficacy for Penicillin and Imipenem respectively on *Listeria monocytogenes* and *P. aeruginosa*. The mechanism involved in this inhibition of bacterial growth is most probably related to a disturbance of the synthesis of the wall. Esinome et al., (2006) have shown that

polyphenols coupled with β-lactams could enhance antibacterial activity by perturbing cell membrane transpeptidation.
Concerning the antibiotics Macrolides, they stop the bacterial proliferation by reversible binding to the 50s subunit of the bacterial ribosome. This interaction inhibits the synthesis of RNA-dependent proteins by preventing transpeptidation and translocation reactions (Hansen et al., 1999), (Zuckerman, 2004). The synergistic effect observed suggests that the extracts combined with Imipenem favor membrane permeability and facilitate antibiotic molecules to reach their intracellular target while contributing to the halting of bacterial multiplication.

Taking into account the results of the extracts on *P. aeruginosa* resistant to Imipenem, we noted that the aqueous extracts are not effective on the inhibition of its growth, but the ethanolic extracts show a remarkable antibacterial capacity and the explanation can be attributed to the texture of its wall provided with an outer membrane rich in phospholipid and forming an impermeable to the hydrophobic molecules (Abi-Ayad et al., 2011). Its resistance to Imipenem is due to a loss of porcine D2 causing a decrease in its permeability, and by a production of chromosomal cephalosporinase (Vurma-Rapp et al., 1990). However, the combination of the four extracts with the Imipenem produced a restoration of the sensitivity of this strain by an improvement of more than 100% of the antibacterial power of this antibiotic. This important finding on the restoration of the weakened efficacy of the Imipenem on *P. aeruginosa,* which is an opportunistic pathogenic bacterium with multi-resistance in continuous evolution, is a beneficial and promising contribution to antibiotic therapy, Therapeutic impasse for certain bacterial strains. The inhibitory effect of the growth of this bacterium is most probably due to the creation of porosity and an increase of the cellular permeability in favor of the entry of the antibiotic. As it can be explained by rupture of other mechanisms of resistance of this germ.

*L. monocytogenes* is the second strain that has revealed this synergistic effect between the two antibiotics Erythromycin and Penicillin. The latter showed an increase in its importante activity of 471% when combined with the two extracts of flower buds and the decoction of the fruits of *C.spinosa*. This bacterial species is thus the most sensitive to the extracts of the different organs with the lowest MICs. This result is due to the low content of its phospholipid wall. The contact of the hydrophobic compounds with the phospholipid bilayer of the bacterial cytoplasmic membrane leads to a failure of the enzymatic system, thus disrupting the ion diffusion and the loss of vital intracellular components (Randrianarivelo et al., 2009).

In the case of the combination of antibiotic Gentamicin with the aqueous extract of fruits against *E. coli* no improvement is observed and its association with the other three extracts on the strains SASM and SARM revealed an antagonistic interaction.

The extracts of *C. spinosa* are known by an amalgam of active compounds in its various parts. Indeed, the phytochemical screening and chemical analysis of the extracts of this plant revealed the presence of polyphenols and its homologues in particular: cappaprenole, flavonoids, hydroxycinnamic acids. It also contains alkaloids, triterpenoids (α-amyrin), sterols and saponins (Satyanarayana et al., 2008). These bioactive phytocomposes inhibit the growth of bacteria and their joint activities with antibiotics lead to an enhanced antibacterial effect and restoration of the antibacterial capacity of antibiotics (Olajuyigbe and Afolayan, 2012).

The mechanism of action of compounds extracted from plants and antibiotics is still poorly illustrated, hence the need for in-depth studies which aim to elucidate their detailed mode of action on pathogenic bacteria.

This technique of combining extracts and antibiotics thus makes it possible, on the one hand, to reduce the dose used by treatment, whose side effects are detrimental to the patient's health, and to solve the problem of bacterial resistance. On the other hand, the management of antibiotics in developing countries is negligible, the high cost of antibiotics and the interruption of medication are consequences of the emergence of multi-resistance bacteria. Therefore, the combination of antibiotics and plant extracts is an opportunity to enhance the effectiveness of weakened antibiotics and reduce the cost of medicating incurable infections.

The interest shown in the results is not only reserved for antibiotic therapy, but it can be of considerable importance in the pharmaceutical, agro-food and cosmetic industries.

## Conclusion:-

According to this study, it is concluded that the ethanolic extracts of the two organs, fruits and flower buds of *C. spinosa* possess more interesting antibacterial activities in vitro than aqueous extracts on multi-resistant pathogenic germs belonging to the Gram-positive and negative.

The combination of the four extracts studied with antibiotics from the beta-lactam and macrolide families shows a synergistic effect.

The results obtained show that these tested extracts inhibit the growth of Gram-positive and Gram-negative bacteria, which allows us to deduce their broadened spectrum of action and exploit the efficacy of their bioactive molecules as adjuvants to antibiotics.

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