Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2015, 7(10):228-236

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

The evaluation of the effect of synthetic flavonoids on growth of pathogenic and probiotic bacteria

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ABSTRACT

*Biological properties of natural flavonoids were succeeding great interest for the synthetic of new flavonoids; recent publications were shown that these last have possessed a various biological activities particularly: antimicrobial, anti-viral, anti-tumoral, and antihypertensive… The aim of this study was to assessment the effect of synthetic flavonoids on growth of probiotic (Lactobacillus rhamnosus and Streptococcus thermophilus) and pathogenic (Staphylococcus aureus and Escherichia coli)bacteria. All tested compounds were exhibited an antibacterial and prebiotic effect; The **7-[3-N-(1,2-O-isopropylidène-α-D-xylofuranos-5-yl)-amino-2-hydroxypropoxyl]-8-méthyl flavone**(product 4) was found to have the most significant activity as antibacterial agents ; Regarding to the prebiotic activity, **7-[3-N-(D, L-glycéryl)-amino-2-hydroxypropoxy]-8-méthylflavone** (product 5) was the most active. The results suggest that these molecules account both the antimicrobial and prebiotic activities.*

Keywords: Flavonoids- antibacterial activity- pathogenic bacteria- probiotic- prebiotic

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INTRODUCTION

Probiotic are microbial dietary adjuvant that beneficially affects the host modulating mucosal systemic immunity as well improving nutritional and microbial balance in the intestinal tract by reduction of its pathogenic members and increasing the potentially beneficial ones (1).The application of probiotic as tools that can be used to manage the GIT ecosystem during development and there by improve the health and nutritionalstatut of the host.The efficacy of these bacteria is dependent on the changes they elicit in the composition and metabolic activities of the resident assemblages of microorganisms. This has encouraged the development of approaches to manage the probiotic bacteria by use of new prebiotic ingredients (2).

Plant is a good source of natural preparations containing effective bioactive compounds which can be used for different applications, as food additives and health promoting ingredients in the formulation of functional foods and neutraceuticals or as prebiotic preparations (3); The major active neutraceutical ingredients in plants are flavonoids; Approximately 2000 individual members of the flavonoids group of compounds have been described. As is typical for phenolic compounds, they can actas potent antioxidants and metal chelators(4). These molecules are a group of organic product ubiquitously distributed in vascular plants, they belong in the family of polyphenol; structurally, this molecules have a common Skelton or core: the flavane nucleus with 15 atom of carbon, consisting of two benzene rings A and B linked by an oxygen-containing pyrane ring (5).

Polyphenolic compounds, particularly flavonoids, are broadly recognized by their various biological activities including antioxidant, anti-inflammatory, anti-allergic, anti-carcingenic, anti-hypertensive and anti-thrombosis (6), (7). These activities are, generally, attribute to their property antioxidant (trap the ROS, chelation of metallic ions and inhibition of the enzymes responsible: xanthine oxydase)*(*4)*;* Flavonoid reactivity with the ROS was the aim of many studies (8).

Interest in antimicrobial activities of flavonoids has increased in recent years; Experimental studies have repeatedly confirm this activities(9), (10), (11); Other studies have put in evidence the impact of flavonoids in the retrovirus HIV of theby the inhibition of reverse transcriptase enzyme of viral DNA replication or affect the adhesion of virus by acting on the glycoprotein of viral surface (12).

From them Alberto *et al* (13), (14), confirm that flavonoids may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect microbial growth according to their constitution and concentration.

Health effects of flavonoids attract the present generation of searchers, particularly their antimicrobial activities; this is why the diversification of flavonoid's source is the major interest. The study of flavonoids chemistry has emerged like that the most natural product, from the search for new compounds with useful physiological properities. Semisynthetic endeavors of oligoflavonoids are in most instances to those substitution patterns exhibited by monomeric natural product that are available in quantities sufficient for preparative purposes. In order to alleviates these restriction, several programs focusing on synthesis of enantiomers pure flavonoids monomers. As for we, we have studied the effect of synthetic flavonoids on bacteria growth which were tested particularly as anti-viral and anti-tumor; The objectif was to demonstrate the application of these flavonoids as tools that can be used to manage the GIT through the evaluation of their effect on probiotic (*Lactobacillus rhamnosus* and *Streptococcus thermophilus)* and pathogenic bacteria (*Staphylococcus aureus* and *Escherichia coli)* activities.

EXPERIMENTAL SECTION

2.1.Microorganism and culture conditions

The pathogenic bacteria *Staphylococcus aureus* was clinical isolate from human vaginal infection and *Escherichia coli* was isolated from a fecal matter of healthy man. The bacteria were identified in our laboratory by their biochemical tests (Gram coloration, API20 and API staph); they were cultured aerobically at 37°C.

Lactobacillus rhamnosus and Streptococcus *thermophiles* were used as probiotic bacteria; *Lactobacillus rhamnosus* was isolated from human fecal matter, and cultured aerobically on MRS; *Streptococcusthermophillus* was isolated from ordinary yoghurt, cultured aerobically on M17.

The probiotic candidate were examined from Gram coloration, catalase activity, oxidase reaction, gas production, sugar metabolism, this tests are confirmed by API strp and API50 CHI; and they were characterized by the principal criteria of selection of probiotic bacteria (Growth at different temperature, at acid pH, resistance to bile salts, anti-bacterial propriety and bacteriocin production, aggregation and adhesion capacity, resistance to antibiotic) **(15)**, **(16)**. The microbial strains were maintained at **-20°C** in culture broth with 15% (v/v) of glycerol **(17)**; prior to any microbial test, the strains were subcultured on fresh appropriate agar plate, incubated for 24h and used as source of bacterial suspension.

2.2. Primary matter used

The molecules used in this study were synthesized as the method of Albert Pare in the Laboratory of Organic Chemistry and Kinetic of Picardie Jules Verne University (Amiens France). These molecules are:

- ✓ 7-glycéryl-8-méthylflavone;
- ✓ 7-O-(D, L-xylityl)-8-méthylflavone;
- ✓ 7-O-(1,2-O-isopropylidène-α-D-xylofuranos-5-yl)-8-méthylflavone;
- ✓ 7-[3-N-(1,2-O-isopropylidène-α-D-xylofuranos-5-yl)-amino-2 hydroxypropoxyl]-8-méthylflavone;
- ✓ 7-[3-N-(D, L-glycéryl)-amino-2-hydroxypropoxy]-8-méthylflavone.

2.3.Antimicrobial and prebiotic effect of natural and synthetic flavonoids

2.3.1. Inoculum preparation

The strains that were frozen at-20°Cwere taken prior to testing, they were inoculated at 37°C for 8 h, then the appropriate agar plats were inoculated from the broth culture, and after incubated at 37°C for 24h the microorganism suspension used for inoculation was prepared according to the density of McFarland (10^7) with broth medium **(18)**.

2.3.2. Anti-microbial and prebiotic tests

Antimicrobial activity and prebiotic effects were evaluated by using the turbidity reader **(19)**, which enables the simultaneous testing of 96 samples and monitoring of bacterial growth in real time during the test.

The broth media used in these tests were: MRS for *lactobacillus rhamnosus*, $M_{17}$for *streptococcus thermophilus* and nutritive broth for *staphylococcus aureus* and *Escherichia coli*. The initial pH adjusted to 6.5 for *lactobacillus rhamnosus* and *Streptococcus thermophilus*; and to 7.0 for *Staphylococcus aureus* and *Escherichia coli*.
The media were inoculated with suspension of 10^7 and incubated at 37°C.

For the preparation of suspension, the cells were harvested from stationary phase culture by centrifugation and suspended in 10 ml of sterile 0.9% 5 (w/v) sodium chloride solutions **(19)**. The CFU counts were based on O.D $_{600nm}$ values **(16)**.

RESULTS AND DISCUSSION

2.1. Antimicrobial effect

The increasing resistance to antibiotic represents the main factor justifying the need to and/or develops new antimicrobial agents. Thus, many studies have been focused on antimicrobial agents and on the antimicrobial properties of plant-derived active principles.

The different results observed after growth of pathogenic bacteria in the presence of synthetic flavonoids showed an inhibitory effect particularly important against *S.aureus* in comparison with control tests.

Figure 1 showed that the inhibitory effect of synthetic flavonoids was important, and these after 38 h of incubation with P_1, 40 h with $P_{2,}$ 42 with P_3; 34h with P_4 and 18h with P_5.

Among the five synthetic flavonoids, P_4 exhibited the highest inhibitory effect on *S.aureus* growth; it showed a decrease of log2 final cell density. The chemical structure of this compound was elucidated that: 7-[3-N-(1, 2-O-isopropylidène-α-D-xylofuranos-5-yl)-amino-2 hydroxypropoxyl]-8-méthylflavone.

Against *E.coli*, the results reported that the synthetic flavonoids affect the growth of *E.coli*; the effect of P_5 was shown weak in comparison with the others P_1, $P_{2,}$ P3 and P4which were showed an important inhibitory effect (figure 2).

In this work, synthetic flavonoids showed an inhibitory effect against *S.aureus* and *E.coli*.

A number studies have confirmed the antimicrobial effect of flavonoids against pathogenic bacteria, particularly against *S.aureus* and *E.coli*; the study of the effect of polyphenols on G + and G- bacteria confirms that these phenolic compounds inhibited *E.coli* and *Salmonella enterica*(9). Also, it has been shown that the flavonoids from propolis extract exhibited an antimicrobial effect against several strains of *E.coli*(10); Another studies reported that pure flavonoids and polyphenolic compounds of different wine were effective against several strains, among it *S.aureus*(11),(20).

On the other hand, the influence of polyphenols on growth and adhesion of commensal *(E. coli*) and pathogenic bacteria (*S. aureus and S. typhimirium*) of human gastrointestinal tract was confirmed(21).

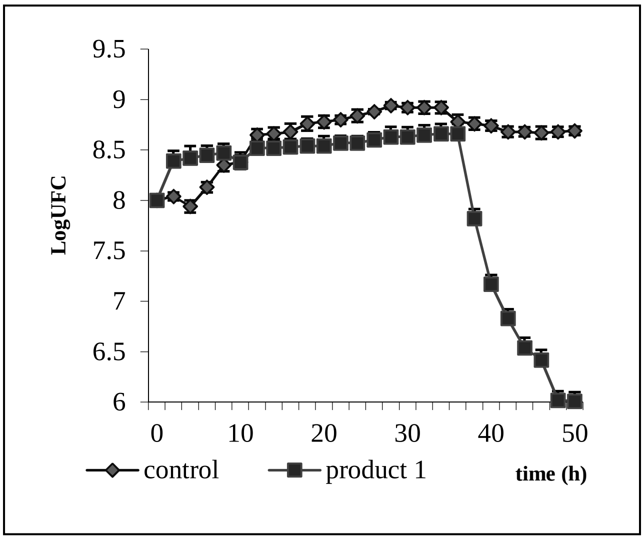
From them, Kuete*et al* (22), confirm the inhibitory effect of four flavonoid, Angusticornin B and Bartericin A prevented the growth of all the pathogens tested, Gancaonin Q and Stipulin were active against 77.27% of the investigated bacterial species, Stipulin and Bartericin A exhibited both antibacterial and anticandidal effects.

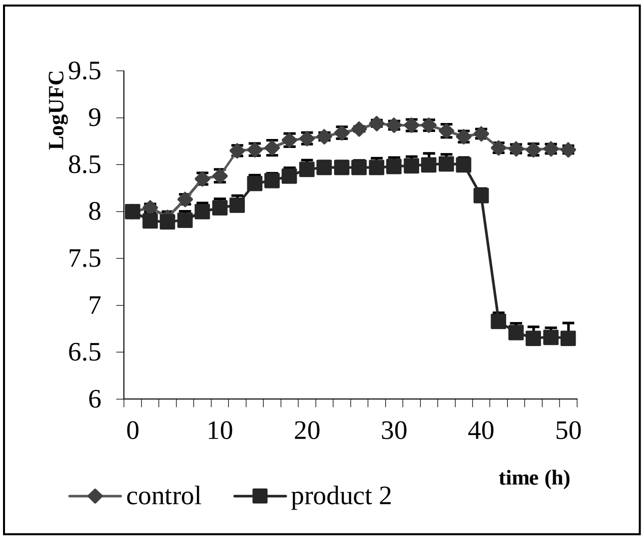
It is important to note that the effect of our compounds was estimated weak in comparison with that of different studies cited; this is may be attributed to the difference of their structures, the high cell concentration (10^7UFC/ ml) cultured in presence of weak concentration of synthetic flavonoids (20%).

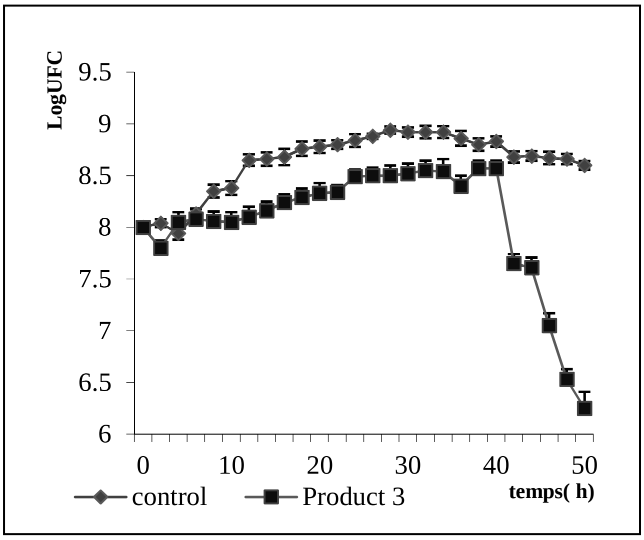
The difference of activities between our five synthetic flavonoids may be attributed to the difference of their chemical structures, several investigation have examined the relationship between flavonoid structure and biological activities, these studies confirms the relationship between the flavonoid structure and their activities (4), (8), (23)and the importance of hydroxyl group on the inhibition of enzymes as xanthine oxydase (3, 3', 4') (24).

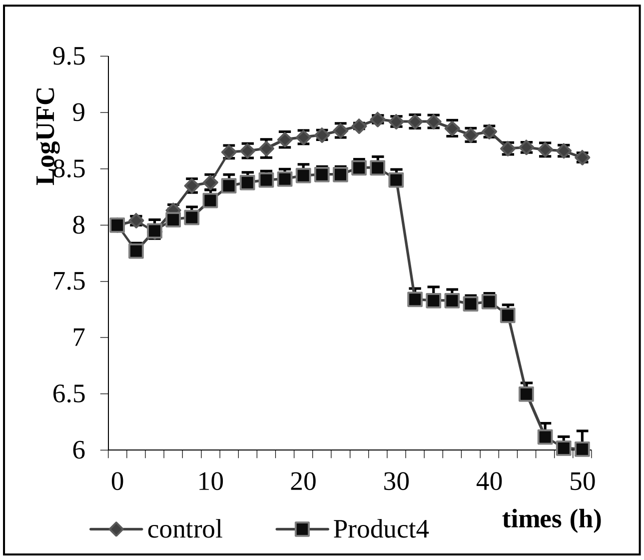
It has been confirmed also that quercitin 3-methyl ether exhibited the highest inhibitory effect on *Helicobacter pylori* than quercitin 3-7-dimethyl ether and kaempferol 3-7-dimethyl ether (25).

The antimicrobial activities of flavonoids can be due to the Inhibition of microbial enzymes, sequestration of necessary substrate or chelation of metal such as iron inhibition of nucleic acid synthesis, inhibition of cytoplasmic membrane functions, membrane disruption, ability to complex with bacterial cell well and adhesives**,** inhibition of cytoplasmic membrane functions or inhibition of energy metabolism.. **(23), (26)**









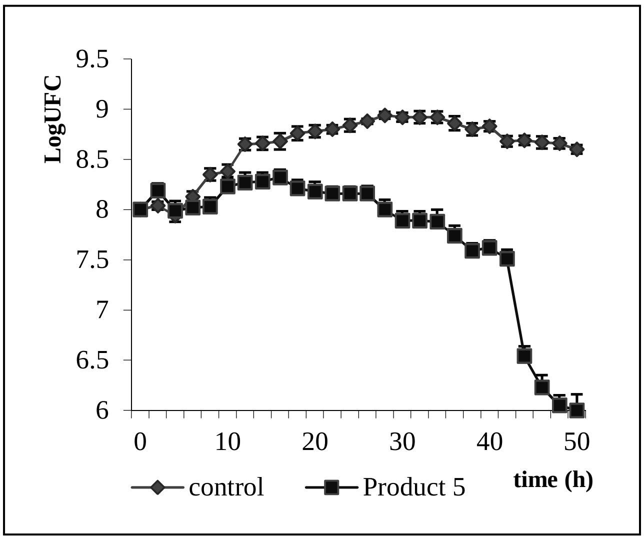


Figure N°01: Effect of synthetic flavonoid on *Staphylococcus aureus* growth. This figure showed the inhibitory effect of flavonoids on the growth of *Staphylococcus aureus*

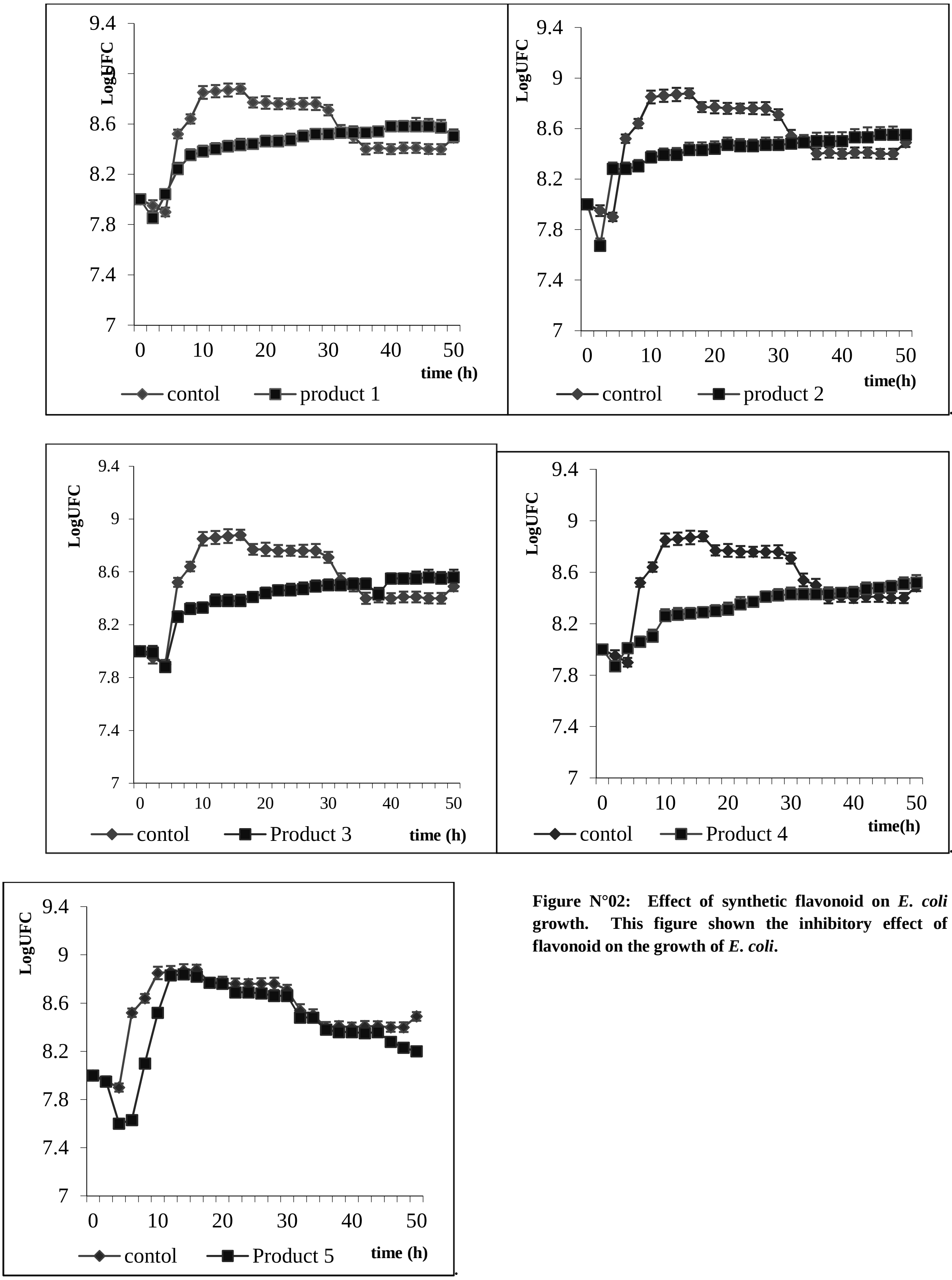
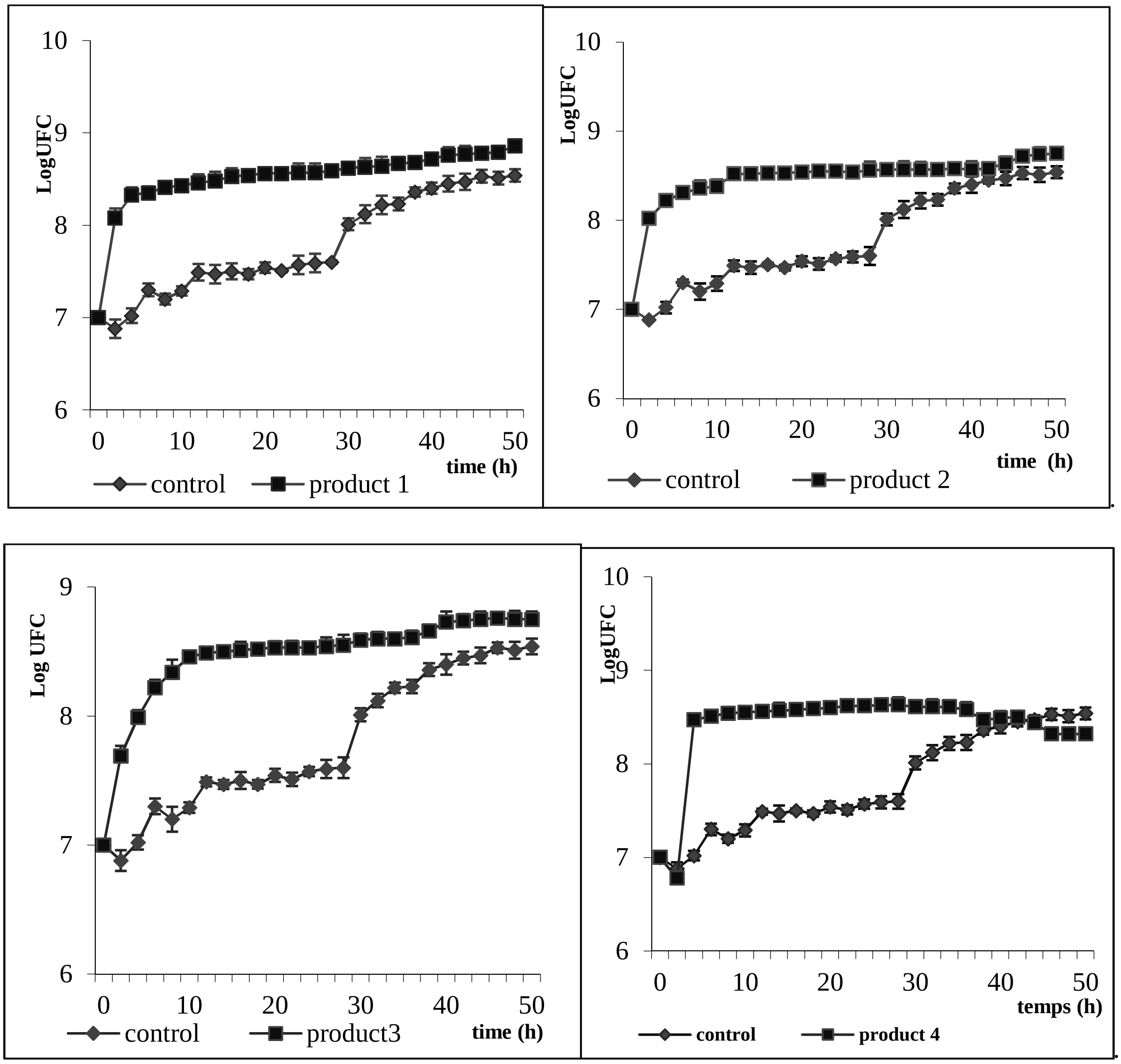


Figure N°02: Effect of synthetic flavonoid on *E. coli* growth. This figure shown the inhibitory effect of flavonoid on the growth of *E. coli*.

3.2. Prebiotic effect synthetic flavonoids

Probiotic bacteria are natural inhabitants of the human gut, they beneficially affect human health by improving the gut microbiota balance and the defenses against pathogens, the growth and the activity of this bacteria can be improved by the prebiotic; the purpose of this test was to evaluate the effect of synthetic flavonoids on the growth of probiotic bacteria.

The effect of synthetic flavonoids on the growth of probiotic bacteria after 50 h of incubation is shown in fig 3 and fig 4.



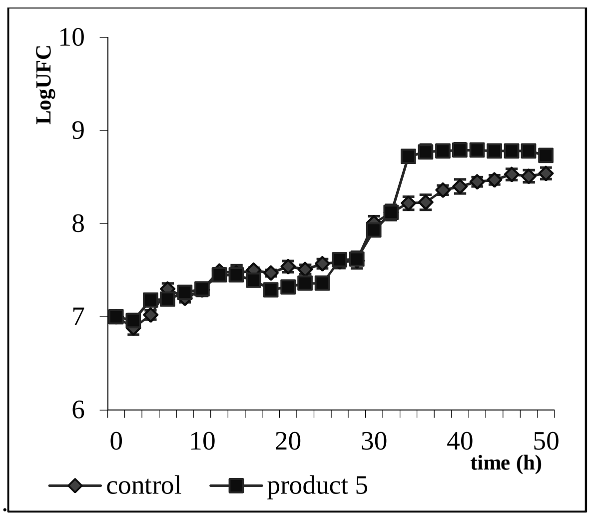


Figure N°03: Effect of synthetic flavonoid on *lactobacillus rhamnosus* growth. This figure showed the stimulatory effect of flavonoid on the growth of *lactobacillus rhamnosus*.

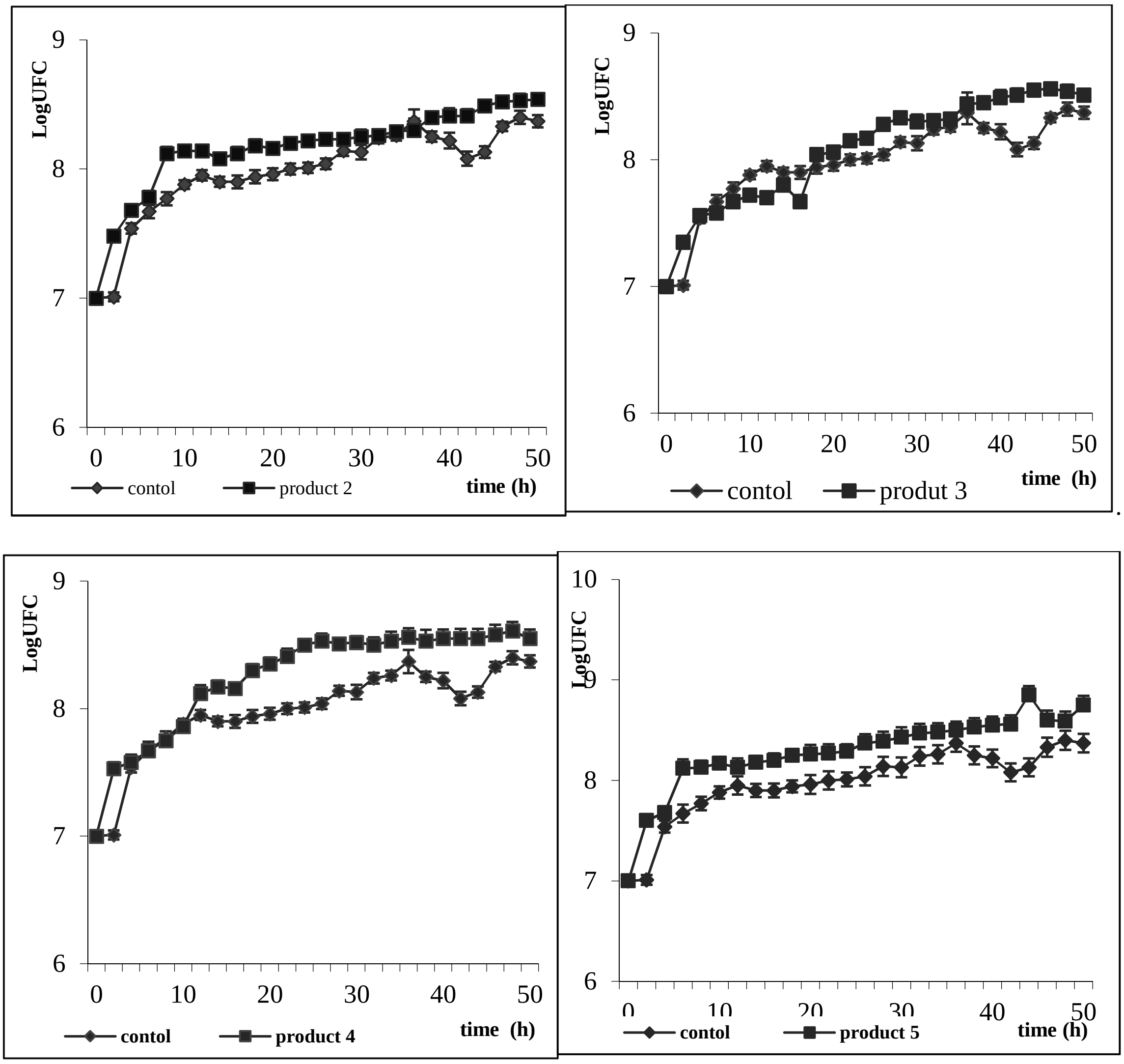


Figure N°04: Effect of synthetic flavonoid on *Sterptococcus thermophillus* growth. This figure showed the stimulatory effect of flavonoid on the growth of *Sterptococcus thermophillus*

Compared to control media (without flavonoids), it can be seen that the growth of *Lactobacillus rhamnosus* is important in the presence of synthetic flavonoids where the growth (LogN) increased from 7 to 8.33 for p1; from 7 to 8.22 for p2; from 7 to 7.99 for p3; from 7 to 8.47 for p4 after 4h of incubation. Among the synthetic flavonoids, p5 showed the weak effect during 32 h; From 34 h this product became more active. Thedifference of activities can be attributed still to the difference of chemical structure.

For *Streptococcus thermophillus*, no significant difference of growth was marked between flavonoids. Furthermore, the stimulatory effect was lower than that noted with *Lactobacillus rhamnosus*. Campos, Couto, and Hogg (27) reported that p-coumaric, caffeic, ferulic, protocatechuic, gallic and vanillic acids present at 100 mg/l did not significantly affect growth of *L. hilgardii*.

Alberto *et al* (13) reported that *L. hilgardii*from wine showed a growth stimulatory effect in the presence of gallic acid and catechin at concentrations normally present in wine.

In their studies Alberto *et al* (14) have demonstrated the ability of *L. hilgardii* X1B to modify the concentration of wine phenolic compounds in culture media, they confirm that the different effect of phenolic compounds on bacterial growth observed among different species and strains of lactic acid bacteria indicate that their effect is strain dependent. Alberto *et al*(28), showed different pH values after the growth of *Lactobacillus hilgardii* in the presence or absence of polyphenolic compounds, in their presence the phenolic compounds have ability to stabilize the pH of media between 4.41 and 4.51.(the initial pH for all media was 4.5). In the other hand, *Parkar et al*(21) demonstrated how several polyphenols, including caffeic acid, catechin, epicatehecin, coumaric acid, phloridzin, rutin, naringenin, daidzein, genistein, and quercetin, enhanced growth and adhesion of *Lactobacillus rhamnosus* to human Caco-2 cells . Several studies were also confirmed the prebiotic effect of pures flavonoids and plant extracts ruched en flavonoids (29), (30), (31).

CONCLUSION

In conclusion, this study argues for the use of flavonoids chemistry to improve the proprieties of the indigenous microflora. Above results demonstrated that the synthetic flavonoids used were showed an antibacterial and prebiotic activities with significant difference where product 4 was found to have the most significant activity as antibacterial agents *and* product 5 as prebiotic substance. In this regard these compounds may be considered as tools that can be used to manage the GIT through the reduction of their pathogenic bacteria and increasing the potentially beneficial ones.

Acknowledgment
This work was realized in Laboratory of bioconversion, health safety and microbiological engineering. University of Mascara, Algeria by the use of flavonoids which synthetized in the Laboratory of Organic Chemistry and Kinetic of Picardie Jules Verne University (Amiens France). The authors wish to thank the staff of laboratoriescited and those responsible.

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