

## Antibacterial activity of Arabic Gum against some pathogenic and non Pathogenic bacteria (Review)

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**Abstract:** Gum Arabic as Prebiotic is food ingredients that stimulate the growth of useful bacteria which lives in the large intestine of the human being or animal since birth and beneficial to the digestive system, body's immunity, disposal of poisons, fats and excreta. Besides it negates the effects of harmful bacteria thus protecting from the diseases, cancer, diabetes and obesity. Present study aimed to investigate the in vitro and in vivo the effect of Arabic gum as exudates and different parts of the tree extracts; Aqueous, ethanol and Hexsane extracts of exudates, leaf, fruits and Bark of Arabic Gum tree as potential antibacterial against different several strains of pathogenic bacteria it was concluded that There was a strong inhibitory effect of aqueous, ethanol and hexane extracts of Arabic Gum exudates, leaf, fruit and bark on most of the gram positive and gram negative bacteria. The largest inhibition zones of bacterial strains were for *Staphylococcus aureus*, followed by *Streptococcus pyogenes* and *Salmonella typhimurium* while the smallest inhibition zones were for *Yersinia pestis*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The three tested strains greatly inhibited due to the presence of Arabic Gum in diet of mice but with different degrees, make us noticed that the most bacterial strain affected was *Streptococcus pyogenes* followed by *Staphylococcus aureus* followed by *Salmonella typhimurium*.

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### 1. Introduction

Wondering what the ingredient called “gum arabic” that’s found in foods like cake, candies, ice cream and soft drinks really is? Gum arabic is a type of plant-derived fiber. You can think of it as an edible “glue,” natural thickening agent and binder that helps hold ingredients together.

Gum arabic’s structure allows it to dissolve in cold or warm water (meaning it’s “water-soluble”), making it easy to use in a variety of ways. Because it is a natural, plant-derived product, it’s suitable for vegans/vegetarians (unlike other products with similar qualities, such as gelatin). It is also naturally gluten-free, usually non-GMO and well-tolerated by most people when used in appropriate/small amounts.

Due to its rich fiber content, gum arabic may offer benefits including increasing probiotic bacteria in the gut, promoting satiety following meals, slowing down gastric emptying and regulating hormone secretion, which helps manage appetite and weight.

All of that said, gum arabic (or acacia gum) is typically found in processed, packaged foods — many of which are high in sugar, low in nutrients and filled with other potentially harmful ingredients. While using gum arabic supplements or baking or cooking with small amounts of gum arabic at home may not be harmful, it’s still best to limit how much packaged food that contains lots of additives you eat in general.

#### What Is Gum Arabic?

Gum arabic, also sometimes called acacia gum or acacia powder, is a fibrous product made from the natural hardened sap of two types of wild *Acacia* trees. Around the world, gum arabic goes by many names, including acacia gum, arabic gum, acacia powder, Senegal gum, Indian gum and others.

*Acacia senegal* (L.), a tree in the *Leguminosae* (*Fabaceae*) plant family, is most commonly used to make gum arabic products. *Vachellia* (*Acacia*) is another species that produces a dried gum from its trunk and branches. These trees grow most abundantly in Sudan, where about 50 percent of the world’s gum arabic is now produced, but are also found in other parts of Africa, such as Kenya, Mali, Niger, Nigeria and Senegal.

What’s interesting about acacia trees is that they produce the most gum arabic when they experience “adverse conditions,” such as poor soil, drought or high heat. This actually damages the trees to some degree but causes an increase in the production of arabic gum.

What type of organic molecule is gum arabic? It is made of a mixture of glycoproteins, a class of proteins that have carbohydrate groups attached to the polypeptide chain, and polysaccharides, a carbohydrate whose molecules consist of a number of sugar molecules bonded together. It also includes oligosaccharides, another type of carbohydrate. Additionally, gums collected from acacia trees are a source of natural sugar compounds called arabinose

and ribose, which were some of the first concentrated sugars to be derived from plants/trees. The exact chemical composition of gum arabic varies from product to product, depending on its source and the climate/soil conditions in which it was grown.

Today, there are many industrial and food-related uses for gum arabic. For example, gelatin, modified starch, gum arabic and pectin are the main types of gums used in many sugary/confectionery products.

Gum Arabic, known as acacia gum, *chaar gund*, *char goond*, or *meska*, is a natural gum made of hardened sap taken from two species of the acacia tree; *Senegalia senegal* and *Vachellia seyal*. The gum is harvested commercially from wild trees throughout the Sahel from Senegal to Somalia, although it has been historically cultivated in Arabia and West Asia. It is a complex mixture of glycoproteins and polysaccharides. It was historically the source of the sugars arabinose and ribose, both of which were first discovered and isolated from it (Renard *et al.*, 2006; Calame, *et al.*, 2008; Rayes, 2013).

Gum Arabic as Prebiotic is food ingredients that stimulate the growth of useful bacteria which lives in the large intestine of the human being or animal since birth and beneficial to the digestive system, body's immunity, disposal of poisons, fats and excreta (Da Silva *et al.*, 2013). Besides it negates the effects of harmful bacteria thus protecting from the diseases, cancer, diabetes and obesity. It is pointed out that the gum-Arabic, extracted from Acacia Senegal trees which is more common in Sudan than anywhere in the world, is one of the natural sources of the prebiotics similar to breast milk and the processed inulin (Gerstenzang *et al.*, 2007; Calame, *et al.*, 2008; Rayes, 2013).

*Acacia arabica* has been proved as effective medicine in treatment of malaria; sore throat (aerial part) and toothache (bark) 3-8 have tested the anti-fertility activity of *A. arabica* pods and nuts. The fresh plant parts of this species have been reported to be most active against Hepatitis C virus (Hussein *et al.*, 2000). It is an important multipurpose tree that has been used extensively for the treatment of various diseases, e.g. colds, bronchitis, diarrhoea, dysentery, biliousness, bleeding piles and leucoderma (Rahaman, 2010). The tree finds use in diabetes, skin diseases and leucorrhoea. These are also used as an antidiarrhoeal, antidysenteric, antidiabetic. The stem bark is astringent, demulcent used in diarrhoea, dysentery, diabetes as astringent, antihelmentic, in skin disease, cough and bleeding piles, gonorrhoea (Siddiqui and Husain, 1993) and as an antiasthmatic (Apparanatham and Chelladurai, 1986). The tender twigs are used as toothbrushes while the thorns are used for joints pains (Tripathi *et al.*, 1982). The gum is used in diarrhoea, dysentery and diabetes (Siddiqui

and Husain, 1991), dry cough in amoebic dysentery, as a tonic, antiasthmatic analgesic and in oral cavity lesions (Sebastian and Bhandari, 1984b). Pharmacologically, GA has been claimed to act as an anti-oxidant, and to protect against experimental hepatic, renal and cardiac toxicities in rats Rajvaidhya *et al.*, 2012.

The antimicrobial activity of the extracts of *Acacia nilotica* was assayed against *Streptococcus viridans*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar diffusion method. The plant extract exhibited antimicrobial activity against all the test microorganisms. *B. subtilis* was the most susceptible to the plant extract while *Candida albicans* was the most resistant (Maslin *et al.*, 2003; Rajvaidhya *et al.*, 2012).

Present review aimed to investigate the in vitro and in vivo the effect of Arabic gum as exudates; Aqueous, ethanol and Hexsane extracts of exudates the exudates, as potential antibacterial against different several strains of some gram positive and some gram negative pathogenic bacteria using the agar well diffusion method.

#### Tested Strains of bacteria:

Strains of pathogenic bacteria were collected and cultured on nutrient broth then cultured each on its specific media as shown in table 1. *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pyogenes*, *Salmonella typhimurium*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Enterococcus faeculis*, *Enterococcus faecium*, *Staphylococcus Epidermidis*, *Streptococcus agalactiae*, *Streptococcus pneumonia*, *Vibrio cholera* and *Yersinia pestis*.

#### Preparation of Arabic gum extracts and experimental solutions:

After obtaining plant (Arabic gum) specimens and the tree parts that will be conducted by the study were processed according to the method. Plant was cleaned and purified of all residues other plant and remove the damaged parts and granulated clay and sand and wash them with water gently to rid it of all impurities and dust. For the purpose of getting the plants in its purest and cleanest form. Dried plant specimens in a well-ventilated room through which passes the air stream with a regular entry of sunlight in the morning with continuous stirring until it was dry.

Preparation of extracts of studied plant part of Arabic Gum tree was selected, dried in sun and finally grinded in electric mill and make the plant part dry fine powder with a different degree of smoothness depending on the part of plant used and the extent of fiber it contains. Some plant specimens used in the study were broken into small pieces then milled using electric grinder for powder preparation of the plant

extracts. Take the constant weights of dry powder samples of plant and put in clean and sterile glass beaker, Added different solvents to get a medical plant extracts are as follows:

1. Water Extract (aqueous extract).
2. Ethanol Extract 70%.
3. Hexane extract.

Beakers were placed on a shaking stirrer for 3 hours then left in room temperature for 24 hours. Plant extracts were filtrated using cotton (Muslin Cloth). Plant extracts re-filtrated by the medical filter paper (Whatman No. (1) For making extract free from any residues Sule and agbabiaka (2008).

**Table 1: Showing investigated pathogenic bacteria with basic laboratory characteristics**

isolate	No of isolates	Gram stain	motility	Specific media
<i>Staphylococcus aureus</i>	ATCC 33591	+ve	Non motile	Enriched media (blood)
<i>Escherichia coli</i>	ATCC 25922	-ve	motile	MacConkey
<i>Enterococcus faecium</i>	--	+ve	Non motile	6.5% Nacl bile-esculin agar
<i>Streptococcus pyogenes</i>	--	+ve	Non motile	Blood agar
<i>Salmonella typhimurium</i>	--	-ve	motile	MacConkey agar
<i>Salmonella typhi</i>	ATCC 14028.	-ve	motile	MacConkey agar
<i>Pseudomonas aeruginosa</i>	ATCC 27853	-ve	motile	MacConkey agar
<i>Klebsiella pneumoniae</i>	ATCC 700603	-ve	Non motile	MacConkey agar
<i>Enterococcus faeculis</i>	ATCC 29212	+ve	Non motile	6.5% Nacl bile-esculin agar
<i>Staphylococcus epidermidis</i>	---	+ve	Non motile	Enriched media (blood)
<i>Streptococcus agalactiae</i>	---	+ve	Non motile	Blood agar
<i>Streptococcus pneumoniae</i>	---	+ve	Non motile	Blood agar
<i>Vibrio cholerae</i>	---	-ve	Rapidly motile	MacConkey Stimulated with Na cl
<i>Yersinia pestis</i>	----	-ve	Non motile	MacConkey

#### Agar well diffusion assay:

The modified agar well diffusion method of Perez *et al.* (1990) was employed. Each selective medium was inoculated with the microorganism suspended in saline solution. Once the agar was solidified, it was punched with a six millimeters diameter wells and filled with 25  $\mu$ L of the plants extracts and blanks (distilled water, ethanol, and hexane). The concentration of the extracts employed was 25  $\mu$ g/ml. Simultaneously, gentamycin sulfate (*S. aureus*, *P. aeruginosa*, *E. coli*), was used as positive controls at a concentration of 1.0  $\mu$ g/ml respectively. The dilution medium for the positive controls was sterile distilled water. The test was carried out by triplicate. The plates were incubated at  $35 \pm 2^\circ\text{C}$  for 24 h. The antimicrobial activity was measured in millimeters.

#### In vitro antagonistic activity of Arabic Gum:

Agar well diffusion technique was used to assess the antimicrobial potential of plant from different sources against investigated gram positive bacteria and gram-negative bacteria. using a sterile swab take the investigated bacterial strain with a concentration of 1.5

x  $10^8$  CFU and spread over a nutrient agar plate and inoculated at  $35^\circ\text{C}$  for 24 h. about 10 $\mu$ l of each extract solution of Arabic Gum was inoculated in wells in the Muller Hinton Agar Medium and incubated at  $37^\circ\text{C}$  for 24 hours. The inhibition zones was measured in mm. and recorded (Perez *et al.*, 1990).

#### In vivo and Challenge experiment (Experimental animals):

A total number of 180 white albino 6-weeks old male mice, they were prepared for the experiment and acclimated for 1 week. They were divided into 5 groups each 10 in three replicates. 1<sup>st</sup> group was subjected skimmed milk with Gum Arabic in a concentration 5mg /200 ml, 2<sup>nd</sup> group was subjected skimmed milk with Gum Arabic in a concentration 10 g/200 ml, 3<sup>rd</sup> group administrated skimmed milk with Gum Arabic in a concentration 15 g/200 ml and 4<sup>th</sup> group administrated skimmed milk with Gum Arabic in a concentration 20 g/200 ml while 5<sup>th</sup> group was used as a control group subjected skimmed milk only without Gum Arabic. All experimental groups administrated skimmed milk with Gum Arabic, two

times daily for 14 successive days before the challenge.

Each mouse in all groups was orally challenged with 0.3 ml of the prepared bacterial suspension ( $1.5 \times 10^8$  CFU/ml) of chosen bacterial strain (the largest three inhibition zones for Arabic Gum) (Silva *et al.* 2004).

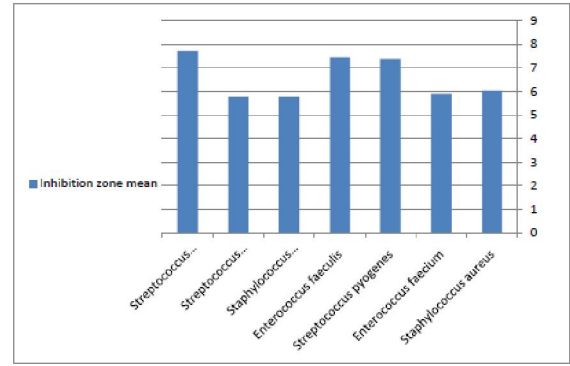
The feces of the mice in each group were individually collected after the administration of the last dose of skimmed milk with Gum Arabic, on the 2<sup>nd</sup>, 5<sup>th</sup> and 10<sup>th</sup> day post infection for detection of the orally administered bacterial strain.

**Fecal colony count:**

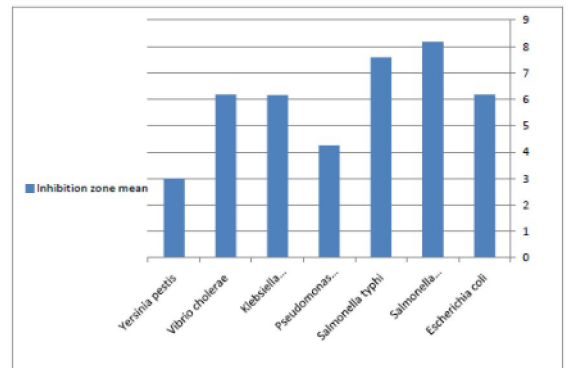
Five grams of Pooled fresh feces were collected from each group separately; feces were diluted in sterile buffered saline (pH 7.2) viable chosen bacterial strain was determined (El-Jakee *et al.*, 2010).

**Basic laboratory characteristics:**

All chosen strains were pathogenic affect the health of human being causing diseases. *Staphylococcus aureus*, *Enterococcus faecium*, *Streptococcus pyogenes*, *Enterococcus faeculis*, *Staphylococcus Epidermidis*, *Streptococcus Agalactiae*, *Streptococcus pneumoniae* are gram +ve, while *Escherichia coli*, *Salmonella typhimurium*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Vibrio Cholera*, *Yersinia pestis* are gram – ve, table 1.



**Fig 2: Showing inhibition zones in mm range and mean for gm +ve bacterial pathogens**



**Fig 3: Showing inhibition zones in mm range and mean for gm -ve bacterial pathogens**

**Table 2: Showing *Staphylococcus aureus* colonies count from fecal samples of challenged mice in the experimental groups**

group	before challenge	2 days	5 days	10 days
gp1 (SM+5 AG)	0	19x10 <sup>4</sup>	5x10 <sup>4</sup> **	3x10 <sup>4</sup> **
gp2 (SM+10 AG)	0	15x10 <sup>4</sup> *	2x10 <sup>4</sup> **	1x10 <sup>4</sup> **
gp3 (SM+15 AG)	0	10x10 <sup>4</sup> *	3x10 <sup>4</sup> **	2x10 <sup>4</sup> **
gp4 (SM+20 AG)	0	7x10 <sup>4</sup> **	3x10 <sup>4</sup> **	0**
Gp6 (SM control)	0	30x10 <sup>4</sup>	26x10 <sup>4</sup>	24x10 <sup>4</sup>

SM=Skimmed Milk AG= Arabic Gum \*significance, \*\*high significance

**Table 3: Showing *Streptococcus pyogenes* colony count from fecal samples of challenged mice in the experimental groups**

group	before challenge	2 days	5 days	10 days
gp1 (SM+5 AG)	0	20x10 <sup>4</sup>	4x10 <sup>4</sup> **	2x10 <sup>4</sup> **
gp2 (SM+10 AG)	0	14x10 <sup>4</sup> *	3x10 <sup>4</sup> **	0**
gp3 (SM+15 AG)	0	9x10 <sup>4</sup> *	3x10 <sup>4</sup> **	1x10 <sup>3</sup> **
gp4 (SM+20 AG)	0	6x10 <sup>4</sup> **	2x10 <sup>4</sup> **	0**
Gp6 (SM control)	0	31x10 <sup>4</sup>	27x10 <sup>4</sup>	22x10 <sup>4</sup>

**Table 4: Showing *Salmonella typhimurium* colonies count from fecal samples of challenged mice in the experimental groups**

group	before challenge	2 days	5 days	10 days
gp1 (SM+5 AG)	0	23x10 <sup>4</sup>	7x10 <sup>4</sup> **	4x10 <sup>4</sup> **
gp2 (SM+10 AG)	0	17x10 <sup>4</sup> *	4x10 <sup>4</sup> **	0**
gp3 (SM+15 AG)	0	12x10 <sup>4</sup> *	5x10 <sup>4</sup> **	1x10 <sup>3</sup> **
gp4 (SM+20 AG)	0	9x10 <sup>4</sup> **	2x10 <sup>4</sup> **	0**
Gp6 (SM control)	0	33x10 <sup>4</sup>	29x10 <sup>4</sup>	24x10 <sup>4</sup>

### Results of antibacterial potency in vivo and Challenge experiment:

All experimental groups administrated skimmed milk with Gum Arabic, two times daily for 14 successive days before the challenge. Pooled fresh feces were collected from each group separately; feces were diluted in sterile buffered saline (pH 7.2) viable chosen bacterial strain was detected and determined (El-Jakee *et al*, 2010). The three investigated bacterial strains undergo the largest inhibition zones; bacterial strains were *Staphylococcus aureus* (6.63-11.54 mm), *Streptococcus pyogenes* (4.45-10.27mm) and *Salmonella typhimurium* (6.33-10.0mm), the investigated strains were effected with orally administrated Arabic Gum but the most group effected was the 4<sup>th</sup> group (SM+20 AG) especially after 10 days. The three tested strains greatly inhibited due to the presence of Arabic Gum in diet of mice but with different degrees, make us noticed that the most bacterial strain effected was *Streptococcus pyogenes* followed by *Staphylococcus aureus* followed by *Salmonella typhimurium* tables 3,4 and 5.

Concerning the effect of gum Arabic in vitro, the present study revealed that the diameter of inhibition zone was taken as an indicator of antimicrobial effect. The present study showed a strong inhibitory effect of Aqueous, ethanol and hexane extracts of Arabic Gum exudates, leaf, fruit and bark on most of the gram positive and gram negative bacteria. The largest inhibition zones of bacterial strains were for *Staphylococcus aureus* (6.63-11.54 mm), *Streptococcus pyogenes* (4.45-10.27mm) and *Salmonella typhimurium* (6.33-10.0mm) while the smallest inhibition zones were for *Yersinia pestis* (1.0-5.0mm), *Pseudomonas aeruginosa* (2.87-5.62mm) and *Staphylococcus epidermidis* (3.83-7.66mm).

Present study use Agar well diffusion test for determination the antibacterial potential of Arabic Gum extracts for different parts of its trees. The results nearly agree with that obtained by Rayes, (2013) and Rajvaidhya *et al.*, (2012) who reported that he antibacterial activity of aqueous extract, different solvent extracts and isolated constituents of were

evaluated by the cup diffusion method against Aqueous, methanol and ethanol extracts of leaves of *Acacia nilotica* (Family: *Fabaceae*) showed significant antibacterial activity against three phytopathogenic *Xanthomonas* pathovars viz., *Xanthomonas axonopodis* pv. *malvacearum*, *X. a.* pv. *phaseoli* and *X. campestris* pv. *vesicatoria* associated with angular leaf spot of cotton, common blight of bean and bacterial spot of tomato respectively and 14 human pathogenic bacteria. This active fraction fractioned from methanol extract recorded highly significant antibacterial activity *in vitro* (MIC 5, 6 and 7 µg/ml for *Xanthomonas* pathovars and 6-12 µg/ml for human pathogenic bacteria) compared with synthetic antibiotics like Bact-805 and K-cycline for phytopathogenic bacteria and Gentamicin and Streptomycin for human pathogenic bacteria (Raghavendra *et al.*, 2006). The mode of action of Arabic Gum extracts as antibacterial till now was unknown but may be due to changing PH of growth due to changing concentration of H ions killing growing pathogenic bacteria.

Regarding results of antibacterial potency in vivo and Challenge experiment present study displayed, all experimental groups administrated skimmed milk with Gum Arabic, two times daily for 14 successive days before the challenge. Pooled fresh feces were collected from each group separately; feces were diluted in sterile buffered saline (pH 7.2) viable chosen bacterial strain was detected and determined (El-Jakee *et al*, 2010). The three investigated bacterial strains undergo the largest inhibition zones; bacterial strains were *Staphylococcus aureus* (6.63-11.54 mm), *Streptococcus pyogenes* (4.45-10.27mm) and *Salmonella typhimurium* (6.33-10.0mm), the investigated strains were effected with orally administrated Arabic Gum but the most group effected was the 4<sup>th</sup> group (SM+20 AG) especially after 10 days. The three tested strains greatly inhibited due to the presence of Arabic Gum in diet but with different degrees, make us noticed that the most bacterial strain affected was *Streptococcus pyogenes* followed by *Staphylococcus aureus* followed by *Salmonella*

*typhimurium*, the obtained results agree with that obtained by Calame, et al. (2008) who reported that the prebiotic efficacy of gum Arabic upon consumption by man for up to 4 weeks. Human healthy volunteers consumed various daily doses (5, 10, 20, 40 g) of gum Arabic (EmulGoldw) in water for up to 4 weeks. Daily consumption of water was taken as the negative control and that of 10 g inulin as the positive control. At 0, 1, 2 and 4 weeks quantification of bacterial numbers in stool samples was performed via real time-PCR techniques and questionnaires were filled in to account for potential drawbacks. The genera of Bifidobacteria and Lactobacilli were taken as potentially beneficial bacteria and those of Bacteroides, Clostridium difficile and Enterococci as potentially non-beneficial, this distinction was dependent on the issue of these numbers being or becoming out of balance in the host. Compared with the negative control the numbers of Bifidobacteria and Lactobacilli 4 weeks after consumption were significantly higher for gum arabic: the optimal dose being around 10 g. Moreover, at this dose the numbers of Bifidobacteria, Lactobacilli and Bacteroides were significantly higher for gum arabic than for inulin. No significant drawback was encountered during the study. It is concluded that gum arabic establishes prebiotic efficacy, at least as good as inulin. The optimal daily dose was found to be 10 g. in addition Da Silva et al. (2013) who reported that Symbiosis between a host and the intestinal microbiota is essential for triggering local and systemic responses favorable to the health of the host. The intestinal microbiota is composed of about 100 trillion bacteria and encompasses more than 1,000 species. It plays an important role in protection against pathogenic microorganisms, development and homeostasis of immune cells, digestion of polysaccharides that is indigestible by human enzymes and fat metabolism, among other functions. and Rayes (2013) who reported that there are symbiosis between prebiotics and probiotics, the results indicated that gum Arabic is good for health as natural prebiotic especially in the larger dose, *Bifidobacterium lactis* Bb12 also good for health and immune status while the combination between gum Arabic as prebiotic and *Bifidobacterium* as probiotic (5 g GA+ Bb12) was better whether the combined application of PRO and PRE (synbiotics) has synergistic or additive effects significantly, they give very good results than each one alone and improve the health conditions, physiological and immune response; cellular and humeral for challenged mice with *salmonella typhimurium*.

From the present review it was concluded that there was a strong inhibitory effect of aqueous, ethanol and hexane extracts of Arabic Gum exudates, leaf, fruit and bark on most of the gram positive and gram

negative bacteria. The largest inhibition zones of bacterial strains were for *Staphylococcus aureus*, followed by *Streptococcus pyogenes* and *Salmonella typhimurium* while the smallest inhibition zones were for *Yersinia pestis*, *Pseudomonas aeruginosa* and *Staphylococcus epidermidis*. The three tested strains greatly inhibited due to the presence of Arabic Gum in diet of mice but with different degrees, make us noticed that the most bacterial strain affected was *Streptococcus pyogenes* followed by *Staphylococcus aureus* followed by *Salmonella typhimurium*.

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