

AN IN VITRO ANTIMICROBIAL ACTIVITY OF *ACACIA SENEGAL* AS PREBIOTIC AGAINST PATHOGENS

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ABSTRACT

An in vitro antimicrobial action of *Acacia senegal* extracts against eleven (11) selected microorganisms was determined using well diffusion technique. The microorganisms with their laboratory number were *Staphylococcus aureus* (MTCC 3160), *Streptococcus pyogenes* (MTCC 1924), *Acinetobacter lwoffii* (MTCC8288), *Proteus vulgaris* (MTCC 742), *Enterobacter aerogenes* (MTCC 2822), *Escherichia coli* (MTCC 739), *Serratia marcescens* (MTCC 7298), *Pseudomonas aeruginosa* (MTCC 741), *Klebsiella pneumoniae* (MTCC 7028), *Saccharomyces cerevisiae* (MTCC 3090) and *Candida albicans* (MTCC 183). Three samples of aqueous extracts of *Acacia senegal* were used in this study. These are; the crude extract and two other processed extracts manufactured by Sattvic foods ltd and Urban platter industry. With the exception of *Serratia marcescens* all the test microorganisms sensitive to all the three aqueous extracts. Ciprofloxacin disc was used as standard and was found to be highly active

INTRODUCTION

Health can generally be regarded as general well-being of an individual, this include physical, psychological and mental state of living organism. World Health Organization (WHO) defined health as state of complete physical, mental and social well-being and not just the absence of disease or infirmity. From medical perspectives, health emphasized on the theme of the body's ability to function normally that can be disrupted from time to time by pathogens. In the ancient time, when a person become ill it would be believed to have been caused by specific deities, because hands symbolized a person's control, and mental illnesses were known as hands of certain deities (Joanne M. W. et al, 2008).

against all the eleven test microorganisms with stronger action against *Serratia marcescens* (inhibition zone of 41.33mm) and the weakest was against *Candida albicans* with an average inhibition zone of 25.67mm.

Key words: *Acacia senegal* (GA), Prebiotics, Probiotics, Bifidobacteria, In vitro, microflora, bifidobacteria, aqueous, hydrocolloids, nosocomial.

As drug resistance cases increases, it became mandatory to search for new and improved agents which are better and without detectable side effects in treating such diseases that are resistance to certain drugs. Hence, the concepts of prebiotics were invented to fight harmful pathogens and boost individual's immune system.

(G R. Gibson and M. B. Robertfroid, 1995) defined prebiotic as non-soluble food ingredients that affect host by selectively stimulating the growth and or activity of certain number of bacteria in host's colon thereby improving the health status of the host. *Acacia senegal* (L) also referred to as *Senegalia senegalia* (Britton) is a legume tree found in the dry tropic and subtropics regions of the world.

In this research, emphasis is made on the use of exudates of *Acacia senegal* as prebiotics against infectious pathogens. The exudates function to seals off the injured tree and prevent the entry of plant pests, pathogens as well as prevent loss of moisture to the surrounding environment.

Gum Arabic, as popularly called is an *Acacia* exudates used in food and drug industries. The phytochemical composition and physical properties of such commercially important *Acacia* gums have been intensively studied (Graefe, 1971).

It also results in over exploitation of resources as products seem to increase the availability of gum Arabic (Lelon, et al, 2010).

Gum Arabic is a derivative of exudates of *Acacia senegal* and is rich in dietary fiber (Nasir et al., 2008), it contains lipoprotein, a heterogeneous gum polysaccharides (Sabahelkhier & Idris, 2010).

Phytochemical analysis of *Acacia* tree leaves revealed the presence of chemicals such as tannin, lignin, pigment, alkaloid, flavonoid, coumarin, naphthoquinone, Saponins, triterpene (Lagnika et al., 2014).

Despite the fact that gum Arabic is widely used as a vehicle for drugs in experimental physiological and pharmacological experiments and is assumed

to be an “inert” substance, some recent reports have claimed that gum Arabic possesses antioxidant, nephroprotectant and other effects (Ali, et al, 2009). Gum Arabic is a complex mixture of polysaccharide consisting of hardened sap of various species of *Acacia* tree. It is also used as emulsifier and a long stabilizer in food and cosmetic products containing oil water interfaces. Gum Arabic is recognized by many researchers that gum Arabic consists of the following three main fractions (Dror, et al, 2006).

1. Polysaccharide (comprising of galactose backbone)
2. High molecular weight arabinogalactan-protein complex (GAGP-GA glycoprotein)
3. A glycoprotein that differs in amino acid composition with larger protein content

The useful properties of gum Arabic are strongly recognized with its structural nature, which determines its solubility, viscosity, its collaboration level with water, its microencapsulation ability as well as its oil in an emulsified state (Montenegro et al., 2009).

Gum Arabic attributes may change fundamentally depending on the age of the trees as well as its geological and geographic origins including location of exudation on the branch of the tree (Masuelli, 2013).

Several species of gum Arabic trees exist in the world, and they differ from one another on the basis of their characteristics and functional properties, (Daoub, et al., 2018).

As an emulsifier and stabilizer, *Acacia senegal* is much more preferred over *Acacia seyal* in international market, and it also has a less colorful solution (Elmanan et al., 2008).

The most stable emulsion among these oils was that of cotton seed, while the least was that of ground nut oil (Patel & Goyal, 2015). *A. senegal* are been sold in market either in processed or mixed form such as Babool shampoo, Nyle shampoo, Ayur shampoo etc (Saini et al., 2008)

Heterogeneous group of natural polymers characterized by their viscosity and dispersion when spread in water (Rayner M. et al., 2016).

Gum Arabic's unique properties together with increasing number of techniques in polymer processing makes it a flexible biopolymer with numerous applications (Ololade, 2018)

MATERIALS AND METHODOLOGY

Materials

Normal saline, Nutrient Agar, Potato Dextrose Agar, Blood Agar, Malt Yeast Agar, Luria Bertani, Sodium Hydroxide (Standard Solution), Methanol, Chloroform, Ciprofloxacin, pH Meter, Test Tubes, Concentrated Hydrochloric Acid, Concentrated Sulphuric Acid, Sodium Bicarbonate, Sensitivity Test Media, Distilled Water.

Equipment

Microscope, micropipettes of varying measure, L- shaped glass spreader, wire loop, petri plates, burner, refrigerator, shaker incubator, beakers, laboratory bottles, laminar hood flow bench.

Sample collection

The raw exudate of the *Acacia* is collected from Rama mandi lay out and some other places around Jalandhar city directly from the injured parts of the tree into specimen bottle.

The sample was handpicked, washed and stored in a clean sterile flat bottom flask with its mouth covered.

Two more processed samples were purchased from amazon store which were manufactured by Sattvic Foods and Urban platter food manufacturing companies. Test microorganisms were obtained from the department of biotechnology laboratory, Lovely Professional University, Phagwara, India.

Sample preparation

Adequate quantity of the exudate was crushed into powdered form using pestle and mortar. It was then seized to fine powder.

50g of each sample were separately weighed and soaked in clean 500ml reagent bottles containing 100ml distilled water and labeled accordingly. The mixture was left to stand for 36hours for complete dissolution.

The mixtures were then sterilized by filtration using a Millipore filter paper of 0.45µm pore size. The pH of the filtrates was measured and stored in a sterile laboratory bottles at 4°C.

McFarland standard no. 2 was prepared by measuring 0.2ml of 1% Barium chloride dihydrate with 9.8ml of 1% Sulphuric acid. This was used to stabilize and lengthen the shelf life the test microorganisms.

Antimicrobial activity determination

Antimicrobial activity of each sample was determined using agar well diffusion method.

Isolate of each test bacteria were tested on nutrient agar plate at 37°C for 24 hours. Similarly, isolates of fungi (*Candida albicans* and *Saccharomyces cerevisiae*) were tested on potato dextrose agar at the same temperature (37°C) for 24 hours.

An inoculum was prepared by transferring few colonies from the cultured test organisms into different test tubes containing normal saline (0.85%). The density of all test microorganisms was adjusted to 10⁶CFU/ml with McFarland standard (2 McFarland).

About 100µl of the inoculum was spread on a freshly prepared media (in triplicate) and spread using L – shaped glass rod spreader.

A well was created on the inoculated media plates using sterile puncture (6mm diameter) and 100µl of the Gum Arabic filtrate was placed in the well.

The inoculated plates were allowed to stand for 60 minutes at room temperature for the filtrates to diffuse. The inoculated plates were then incubated overnight at 37°C.

After 24hours incubation, the plates were observed for inhibition zones. The inhibition zone of each plate was measured and mean of each triplicate was determined.

Antimicrobial Activity Assay Determination

Antimicrobial activity assay of the test organisms were determined using agar disc diffusion method. The agar plates (nutrient agar in case of bacteria) and potato dextrose agar (in case of fungi) were inoculated with about 0.1ml of the test microorganisms and spread evenly with the aid of sterile L- shaped glass rod. Ciprofloxacin discs were placed in the center of each plates containing the inoculum (the plates were in triplicates) and incubated overnight. After the incubation, zone of inhibition were noted and recorded for each isolates.

The ciprofloxacin was used as standard for the antimicrobial as well as antifungal activity test.

Table 1: Test organisms used in study

Gram negative bacteria	Gram positive bacteria	Fungi
<i>Acinetobacter lwoffii</i>	<i>Staphylococcus aureus</i>	<i>Saccharomyces cerevisiae</i>
<i>Proteus vulgaris</i>	<i>Streptococcus pyogenes</i>	<i>Candida albicans</i>
<i>Enterobacter aerogenes</i>		
<i>Escherichia coli</i>		
<i>Serratia marcescens</i>		
<i>Pseudomonas aeruginosa</i>		
<i>Klebsiella pneumonia</i>		

Table 2. Physical nature of *Acacia senegal* powder used

Extract sample	Texture	Color
Crude	Crystal	Orange brown
Sattvic	Fine	White
Urban platter	Fine	Creamy

Table 3. PH value of the extracts filtered and normal saline solution

Extracts	pH value
Crude	4.22
Sattvic	4.28
Urban platter	4.08
Normal saline	7.91

RESULT AND DISCUSSION

The antimicrobial activity of *Acacia senegal* was determined against some selected bacteria and fungi (table 1). Nine (9) were bacteria while two (2) were fungi.

Three different forms of aqueous *Acacia senegal* extracts were used in this study. These are the crude extract and two other processed gum Arabic manufactured by Sattvic Foods and Urban platter industry.

This study revealed the distinguishing properties of both three extracts based on physical appearances (table 2)

The antimicrobial actions of these extracts were assessed with respect to zones of inhibition as shown in fig. 1, 2 and 3. **As prebiotic, the results obtained shows that gum Arabic exhibit antimicrobial actions against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Acinetobacter lwoffii*, *Proteus vulgaris*, *Enterobacter aerogenes*, *Escherichia coli*, *Serratia marcescens*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Saccharomyces cerevisiae* and *Candida albicans*. However, the following were observed the Crude gum Arabic did not show antimicrobial action against *P. vulgaris*, *S. marcescens* and *S. cerevisiae*, Sattvic extract did not show antimicrobial action on *A. lwoffii* and *S. marcescens* and similarly *A. lwoffii*, *P. vulgaris* and *S. marseceus* are not sensitive to Urban platter aqueous extract (table 4).**

The degree and strength of antimicrobial actions of each aqueous extract is determined by the size of inhibition zone produced by the extracts (table 4 and 5). The larger the size of the zone of inhibition, the higher the antimicrobial action of the extracts. **The objectives of this work are to study and evaluate the antimicrobial activity of *A. senegal* as prebiotic against some selected microorganisms. The study shows that all the three aqueous extracts show varying degree of antimicrobial action against the selected microorganisms.** With the exception of *Serratia marcescens* and *Saccharomyces cerevisiae*, the entire selected microorganisms are sensitive to all the three gum Arabic extracts (table 4). **The weakest sensitivity is shown by extract of Urban platter extract against *Pseudomonas aeruginosa* with mean inhibition zone of 7.66mm, while the strongest sensitivity is shown by same Urban platter extract on *Enterobacter aerogenes* with mean inhibition zone of 15.33mm (table 5).** Similarly, ciprofloxacin disc was employed to check its antimicrobial action against all the test organisms.

In this study, ciprofloxacin displayed a great antimicrobial action against the entire test organism. With the strongest action against *Serratia marcescens* with

an average inhibition zone of 41.33mm, while the weakest antimicrobial action was against *Candida albicans* having an average inhibition zone of 25.67mm.

Table 4: Positivity of each *Acacia* samples against each test organism

Test Organisms	Gum Arabic extract sample used			
	Crude GA	Sattvic GA	U/platter GA	Ciprofloxacin
<i>Staphylococcus aureus</i>	+	+	+	+
<i>Strept. pyogenes</i>	+	+	+	+
<i>Acinetobacter lwoffii</i>	+	-	-	+
<i>Proteus vulgaris</i>	-	+	-	+
<i>Ent. aerogenes</i>	+	+	+	+
<i>Escherichia coli</i>	+	+	+	+
<i>Serratia marcescens</i>	-	-	-	+
<i>Ps. aeruginosa</i>	+	+	+	+
<i>Kleb. pneumonia</i>	+	+	+	+
<i>S. cerevisiae</i>	-	-	-	+
<i>Candida albicans</i>	+	+	+	+

Table 5: Inhibition zones recorded for each *A. senegal* extract against each test organisms and Ciprofloxacin disc.

Test organisms under study	Mean inhibition zone of each GA extract and Ciprofloxacin disc (mm)			
	Crude GA	Sattvic GA	U/Platter GA	Ciprofloxacin
<i>Staphy. aureus</i>	9.00	9.3	8.6	30.3
<i>Strept. pyogenes</i>	9.33	8.6	11.00	25.6

		7		7
<i>Acinetobacter lwoffii</i>	12.00	10.67	10.33	38.33
<i>Proteus vulgaris</i>	10.33	0.00	0.00	39.33
<i>Ent. aerogenes</i>	9.00	9.00	15.33	29.33
<i>Escherichia coli</i>	9.33	10.33	9.33	28.66
<i>Serratia marcescens</i>	0.00	0.00	0.00	41.33
<i>Ps. aeruginosa</i>	8.33	9.33	7.66	26.00
<i>Kleb. pneumonia</i>	13.33	9.33	13.66	29.66
<i>S. cerevisiae</i>	0.00	0.00	0.00	25.66
<i>Candida albicans</i>	14.33	11.00	12.66	25.67

Table 6: Sensitivity strength of each *Acacia* sample against each test organism

Test Organisms	Gum Arabic extract sample used			
	Crude GA	Sattvic GA	U/platter GA	Ciprofloxacin
<i>Staphy. aureus</i>	Weak	Weak	Weak	Strong
<i>Strept. pyogenes</i>	Weak	Weak	Weak	Strong

<i>Acinetobacter lwoffii</i>	Moderate	Weak	Negative	Strong
<i>Proteus vulgaris</i>	Weak	Moderate	Negative	Strong
<i>Ent. aerogenes</i>	Weak	Weak	Moderate	Strong
<i>Escherichia coli</i>	Weak	Weak	Weak	Strong
<i>Serratia marcescens</i>	Negative	Negative	Negative	Strong
<i>Ps. aeruginosa</i>	Weak	Weak	Weak	Strong
<i>Kleb. pneumonia</i>	Moderate	Weak	Moderate	Strong
<i>S. cerevisiae</i>	Negative	Negative	Negative	Strong
<i>Candida albicans</i>	Moderate	Weak	Moderate	Strong

Key: 7mm to 12mm = Weak, 12.1mm to 20mm = Moderate above 20mm = Strong

Table 7 Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	CGA	8.6345	11	4.68107	1.41140
	SGA	7.0600	11	4.58905	1.38365
Pair 2	CGA	8.6345	11	4.68107	1.41140
	UPGA	8.0573	11	5.62310	1.69543
Pair 3	CGA	8.6345	11	4.68107	1.41140
	C	30.9064	11	5.92092	1.78522
Pair 4	SGA	7.0600	11	4.58905	1.38365
	UPGA	8.0573	11	5.62310	1.69543

Pair 5	SGA	7.0600	II	4.58905	1.38365
	C	30.9064	II	5.92092	1.78522
Pair 6	UPGA	8.0573	II	5.62310	1.69543
	C	30.9064	II	5.92092	1.78522

Table 8 Paired Differences between extracts

S/N	Extract type	Number of organism	Mean SD	SEM	t- value	P' value
1	Crude GA	II	3.32		1.56	0.148
	Sattvic GA					
2	Crude GA	II	3.90		0.49	0.639
	U/P GA					
3	Crude GA	II	8.12		9.08	0.00
	Ciprofloxacin					
4	Sattvic GA	II	2.45		1.34	0.208
	U/P GA					
5	Sattvic GA	II	8.99		8.79	0.00
	Ciprofloxacin					
6	U/P GA	II	9.68		7.69	0.00
	Ciprofloxacin					

Plants with antimicrobial activities represent a large source of drugs and drugs related properties which require continue exploration for the **treatment of various ailments**.

This study reveals that the aqueous extracts of crude gum Arabic and those of the processed gum Arabic (from Sattvic Foods ltd and Urban platter) have an inhibiting effects on the growth and development of all the tested microorganisms except *Saccharomyces cerevisiae*, and this work is supported by the work done on the aqueous extract that demonstrated antimicrobial actions against bacteria and fungi (Clark et al, 1993).

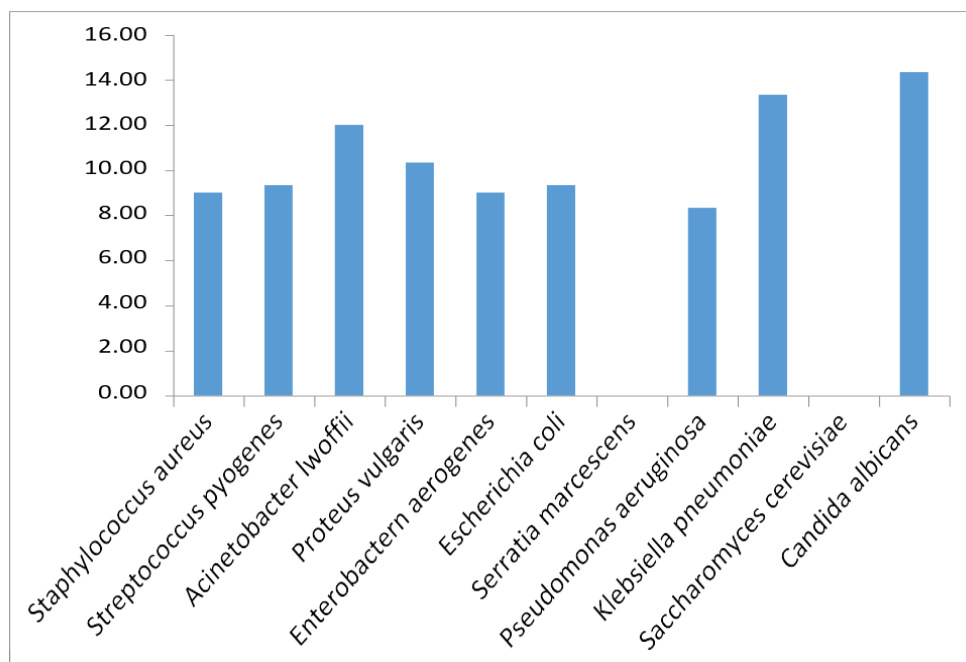
The strains of gram positive and gram negative bacteria as well as the fungal strains used in this research work have similar susceptibility.

Available literature has it that most antimicrobial effects of gum Arabic was due to its secondary metabolites or due to the presence of certain phytochemicals which includes Saponins, volatile oil, tannins, flavonoids, phenols, alkaloids and terpenes (Saurabh et al., 2012).

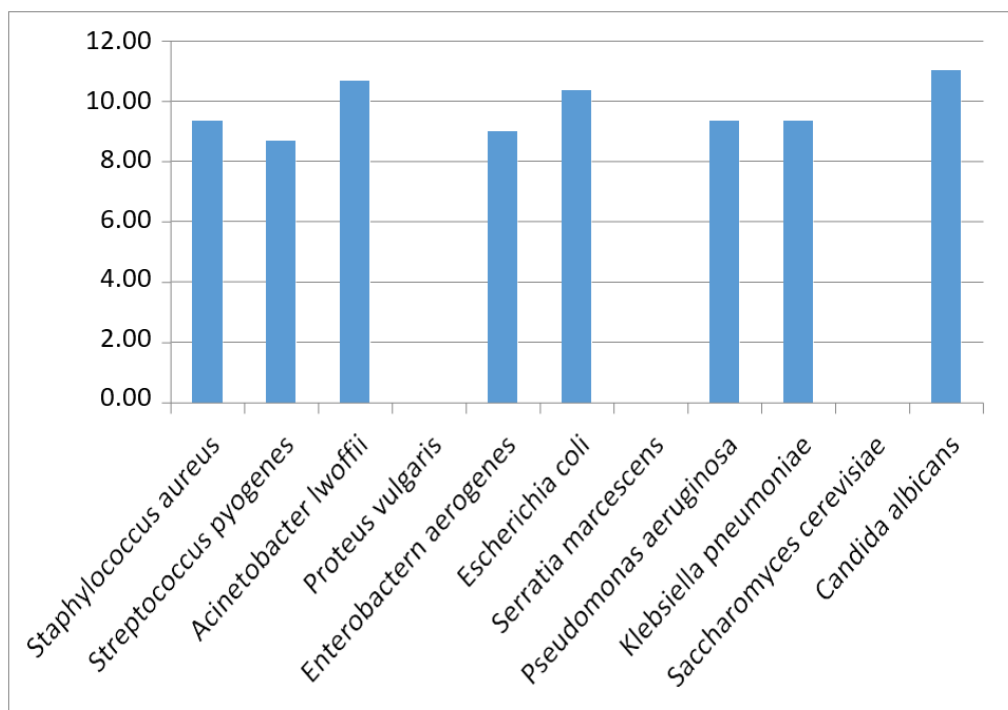
The antimicrobial activities of the gum Arabic used in this study could be attributed to the presence of terpenes.

Terpenes represent a large group of biologically active hydrocarbons present in plants oil.

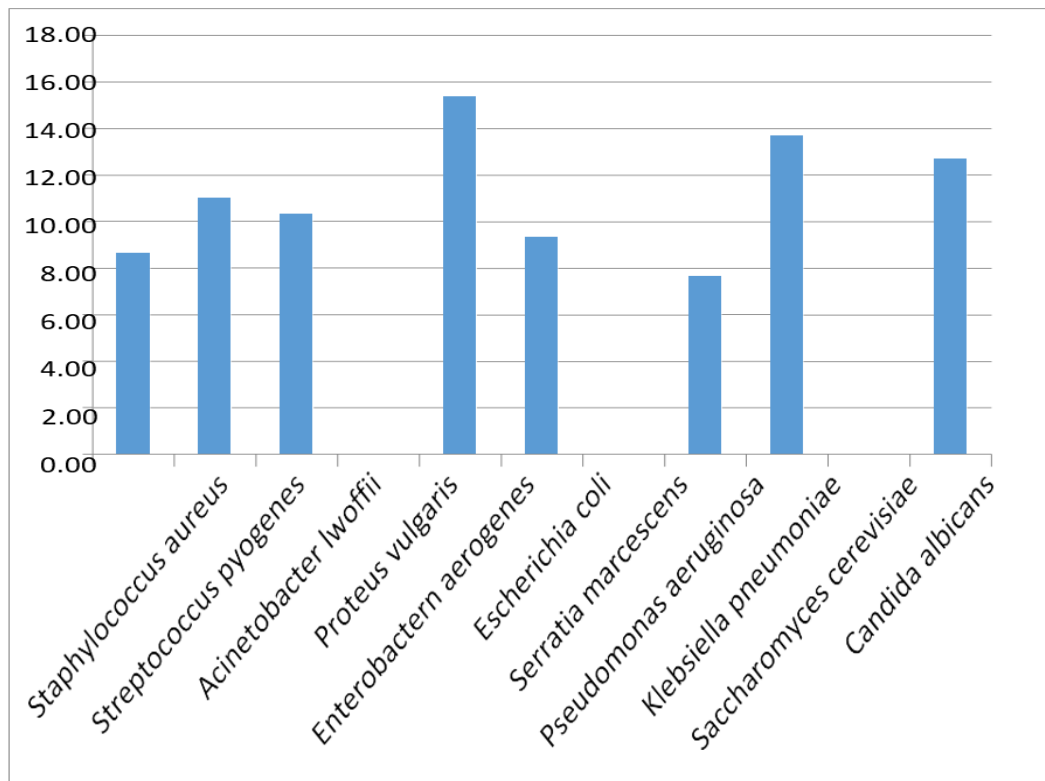
Terpenes serves as the plants' defense system (just like white blood cells in animals), as such it is equally referred to as phytoprotectants. Many terpenes are obtained from conifer resins. Most common constituents of oils of most plants and flowers are terpenes and terpenoids. Available literature also has it that gum Arabic contains salts such as Mg^{2+} , Ca^{2+} and K^{2+} whose effects were attributed. So, also gum Arabic is known to possess certain enzymes such as peroxidases, pectinases and oxidases as well among which others are known to have antimicrobial potentials.



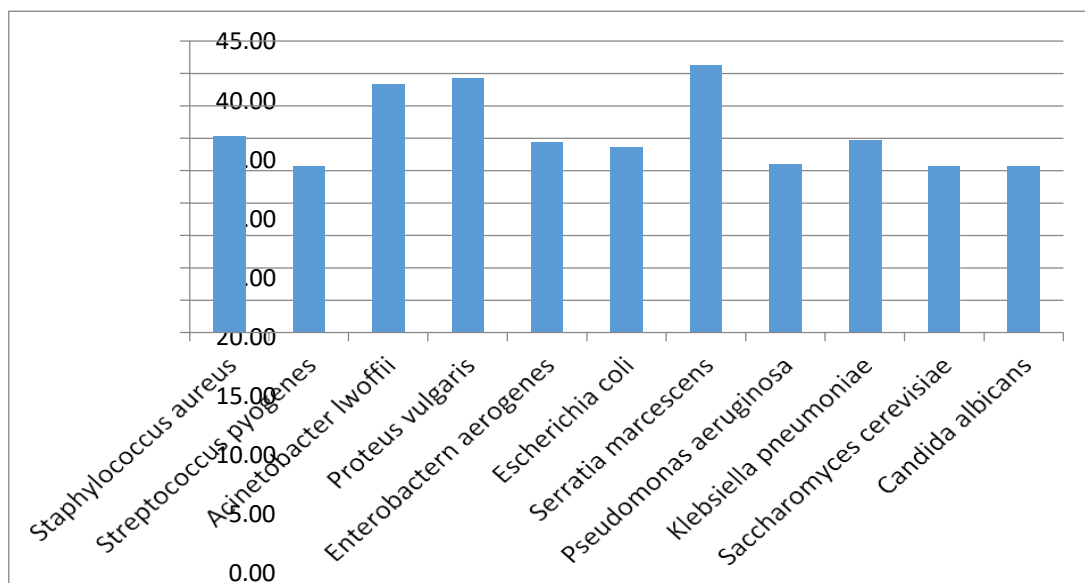
Graph 1. Antimicrobial Activity of crude extract of *A. senegal* against test microorganisms



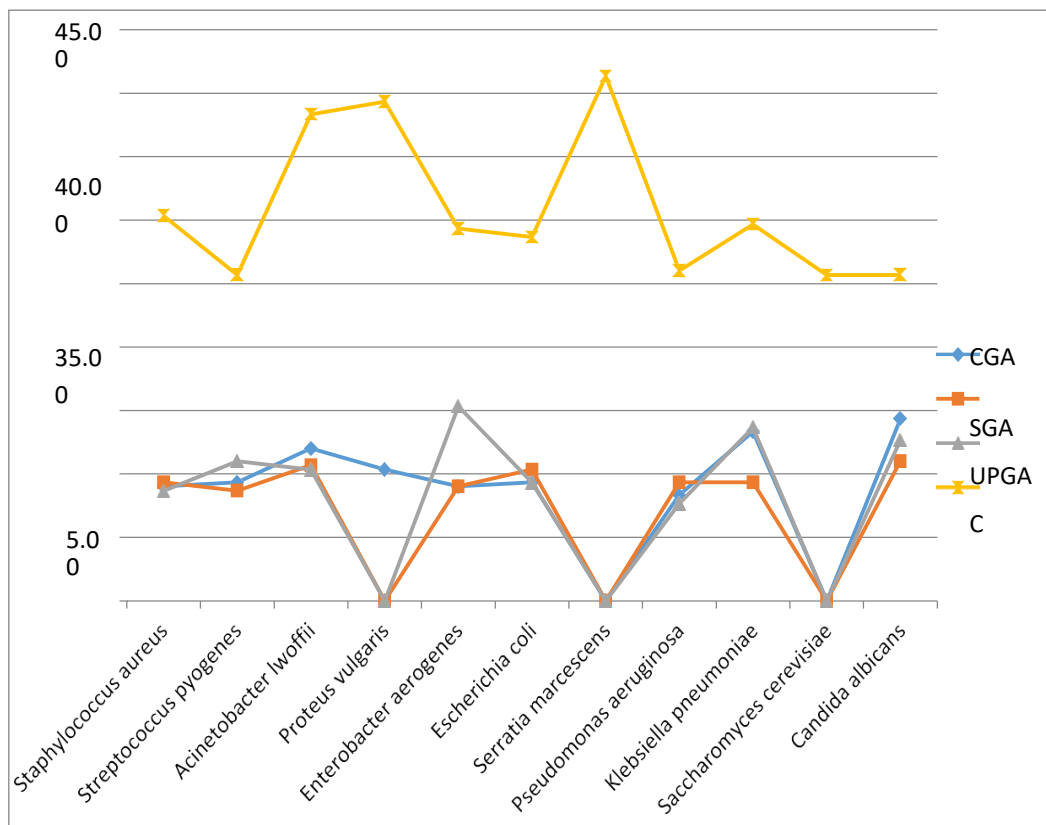
Graph 2. Antimicrobial Activity of Sattvic *A. senegal* extract against test microorganisms



Graph 3. Antimicrobial Activity of Urban platter *A. senegal* extract against test microorganisms



Graph 4. Antimicrobial Activity of Ciprofloxacin disc against test microorganisms



Graph 5 Paired Samples T – test Graph

DISCUSSION

Due to its numerous advantages and lesser side effects, excellent tolerability, its availability and worldwide acceptance, plants and plant products are getting attention and popularity since ancient time, thereby providing meaningful treatment of diseases not curable by some other means. The aim of this work is to study the in vitro antimicrobial potential of *Acacia senegal* aqueous extracts against some selected bacteria and fungi of economic importance.

This study shows that all the three aqueous extract of the *Acacia senegal* has significant importance as herbal medicine.

Parts of gum Arabic tree were reported by some researchers of repute to have been in usage by the rural people in the treatment of certain ailments such as sexual weakness in males, diarrhea, gonorrhea, menstrual discomfort and dysentery among others. Seeds of gum Arabic are used in the treatment of skin related problems and stomach disorder (Saini et al., 2008).

Traditional usage of other gum Arabic species such as *Acacia nilotica*, *Acacia*

catechu in the management and treatment of digestive disorder, treatment of sexually transmitted diseases such as syphilis and gonorrhea, treatment of cholera, leprosy and dysentery, chest pain, sores as a result of cancer, toothache, cough as well as asthma were also reported (Chopra et al., 1956).

CONCLUSION AND RECOMMENDATIONS

Considering the findings in this study, it was concluded that aqueous of *Acacia senegal* has demonstrated certain degree of antimicrobial action against the selected microorganisms investigated, thereby offering an bountiful opportunity to formulate plant based drugs due to its greater efficacy against economically important pathogens.

This study further support the evidences that support the idea that *Acacia* genus plants is among plants genus that are rich in bioactive compounds with promising drugs properties.

Due to its antimicrobial actions, *Acacia senegal* has been recommended by several studies to be an all effective therapy for the treatment of many ailments including lowering blood pressure, reducing the concentration of plasma phosphate and decreasing the total plasma cholesterol as well.

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