DETECTING THE ANTI-STREPTOCOCCUS PYOGENS ACTIVITY OF CYANOBACTERIUM *(APHANOCAPSA SPECIES)* BY USING PLACKETT-BURMAN EXPERIMENTAL DESIGN

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ABSTRACT

Streptococcus pyogenes is associated with a wide range of infections and disease states, that infects children and adolescents causing sore throat. This study was carried out to evaluate the potential of exopolysaccharides of *Aphanocapsa sp* was tested for antibacterial activity against *Streptococcus pyogenes* (Group A streptococcus) bacterial cultures using well diffusion method.Plackett- Burman design was applied on original BG11medium to determine the best conditions that effect on activity of exopolysaccharides of *Aphanocapsasp* against *Streptococcus pyogenes***.** Plackett- Burman design was performed on original BG11medium by applied various nitrogen sources $NaNO_3$and ammonium chloride. It was found that exopolysacchaaride extracted from *Aphanocapsa sp* had activity against *Streptococcus pyogenes*. Exopolysacchaaride extracted from alga that grown in Bg11medium supplemented with ammonium chloride had more effective against *Streptococcus pyogenes* than that obtained from Bg11medium supplemented with $NaNO_3$.

Key Words : Antibacterial , *Aphanocapsa sp***.** ,*Streptococcus pyogenes* **,** cyanobacteria **,** Plackett- Burman design**.**

INTRODUCTION

Streptococcus pyogenes diseases remain a major public health problem in developing countries, reaching 600 million cases per year and thus constituting an important cause of morbidity and mortality (PUB MED) . Group A streptococci (GAS) infections can lead to severe invasive diseases including pharyngitis and pyoderma and to autoimmune post-streptococcal sequelae, such as rheumatic fever (RF) and glomerulonephriti (Wikipedia).This increase of antibiotic resistant bacteria is a serious issue because of the constant concern of reduced efficiency of antibiotics in the treatment of human diseases (Chandruet al 2013).Isolation of bioactive compounds from cyanobacteria is done with two objectives: one is to discover new compounds for pharmaceutical, agricultural or biological application; the other is for the better understanding of the interactions of individual organisms within their natural communities. For each of these

purposes, there is a need to screen new organisms (Rania and Hala, 2008). The ability to produce antimicrobial substances may be attributed to the defensive nature to survive in different habitats of the species and also a good source of new bioactive compounds (Rania and Hala, 2008). Cyanobacteria, known as blue-green algae include a highly diverse group of prokaryotic microorganisms and widely distributed in nature and can be found in most terrestrial and freshwater habitat (Potts, 2002). Cyanobacteria is considered to be one of the potential organisms and useful to mankind in various ways. A number of important advances have occurred in cyanobacterial biotechnology in the recent years.(RizviRimsha et al.2014). Cyanobacteria produce many bioactive compounds, both intra- and extracellular to

survive in extreme environmental sources (Dvornyk and Nevo, 2003;Kulik, 1995; Kreitlow et al.,1999; Patterson et al.,1994). Recently, microalgae have become particularly interesting because of the possibility to easily control the growth conditions in a bioreactor together with the demonstrated biochemical diversity of these organisms (Akmjmop et al., 2015). Greater screening and selection efforts for biologically active compounds, including polysaccharides, have been developed (DePauw and Persoone, 1988). Exopolysaccharides in pharmaceutical industry they can be used as antiviral (Hayashi et al., 1996 a, b; Singh and Das, 2011), Microbial Exopolysaccharides (EPSs) are

biosynthetic polymers mainly consisting of carbohydrates secreted by bacteria (Freitaset et al., 2009) and cyanobacteria (Parikh and Madamwar, 2006).

MATERIALS AND METHODS

Cyanobacterial strain isolation and identification:

Cyanobacterial strain was isolated from cultivated rice fields in ElGharbia district,Egypt.Culture purification was according to Andersen (2005), Van Landingham and Collins (1982).

Culture conditions:

*Aphanocapsasp*was grown in axenic cultures at 28±2°C. under continuous illumination (50 µmol photons m-2 s-1) in 500 mL conical flasks, containing 200 mL BG11media (Steiner et al 1971) then to apply Plackett–Burman design.

Firstly: Total eight variables from BG-11 were screened include $NaNO_3$, K_2HPO4, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, citric acid, ferric ammonium citrate, EDTA and Na_2CO_3.

Secondly : thePlackett–Burman design was applied withNH_4Cl as nitrogen source instead of $NaNo_3$.

Plackett–Burman (PB) designs are a class of fractional factorial designs first developed by two mathematicians/statisticians (Plackett and Burman 1946) A Plackett-Burman, which is traditionally used for identifying important factors from many potential factors.

Table 1.Effect of the components of BG-11 medium on algal growth using the Plackett-Burman multifactorial design.

Trial	Level and concenteration of variable (g/l)							
	X1 $NaNo_3$	X_2 K_2HPo_4	X_3 $MgSo_4.7H_2O.$	X_4 $CaC_2.2H_2O$	X_5 Citric acid	X_6 Ferric ammoniu m citrate	X_7 EDTA	X_8 Na_2Co_3
T_1	+2.25	-0.02	+0.1125	-0.018	-0.003	-0.003	+0.0015	+0.03
T_2	+2.25	+0.06	-0.0375	+0.054	-0.003	-0.003	-0.0005	+0.03
T_3	-0.75	+0.06	+0.1125	-0.018	+0.09	-0.003	-0.0005	-0.01
T_4	+2.25	-0.02	+0.1125	+0.054	-0.003	+0.09	-0.0005	-0.01
T_5	2.25	+0.06	-0.0375	+0.054	+0.09	-0.003	+0.0015	-0.01
T_6	+2.25	+0.06	+0.1125	-0.018	+0.09	+0.09	-0.0005	+0.03
T_7	-0.75	+0.06	+0.1125	+0.054	-0.003	+0.09	+0.0015	-0.01
T_8	0.75	-0.02	+0.1125	+0.054	+0.09	-0.003	+0.0015	+0.03
T_9	-0.75	-0.02	-0.0375	+0.054	+0.09	+0.09	-0.0005	+0.03
T_{10}	2.25	-0.02	-0.0375	-0.018	+0.09	+0.09	+0.0015	-0.01
T_{11}	-0.75	+0.06	-0.0375	-0.018	-0.003	+0.09	+0.0015	+0.03
T_{12}	-0.75	-0.02	-0.0375	-0.018	-0.003	-0.003	-0.0005	-0.01
T_{13}	1.5	0.04	0.075	0.036	0.006	0.006	0.01	0.02

Exopolysaccharide (EPs) extraction: After culture centrifugation (4,500 g, 10 min) the EPS was precipitated by an equal volume of isopropanol, filtered and dried at 37°C (Reddy *et al*., 1996; Pawar*et al*., 2013).

Screening of anti-*Streptococcu spyogenes* (ATCC 19615) activity of *Aphanocapsa sp*.

This was done by using well diffusion method on blood trypticase soy agar medium andaccording to (Chandru et al 2013) . The diameter of inhibition zone around each well was measured which shows non hemolysis (non rupturing of red blood cells) While The rest ofthe plate shows beta hemolysis (complete rupturing of red blood cells) visible as a halo in culture.

RESULTS AND DISCUSSION

In order to find out the key ingredients significantly affecting bioactivity and biomass production, a Plackett-Berman design was carried out and Minitab 16 software used to analyze the results.

Effect the components of BG-11 medium on algal growth using $NaNO_3$ as nitrogen source

Table 2. Estimated effects and coefficients for analysis of Plackett–Burman design on EXP g/L

Term	Effect	Coef	SE Coef	T	P
Constant		0.00000	0.01263	0.00	1.000
$NaNo_3$	-0.00000	-0.00000	0.01263	-0.00	1.000
K_2HPo_4	-0.00000	-0.00000	0.01263	-0.00	1.000
$MgSo_4.7H_2O$	-0.00000	-0.00000	0.01263	-0.00	1.000
$CaCl_2.2H_2O$	-0.00000	-0.00000	0.01263	-0.00	1.000
Citric acid	-0.00000	-0.00000	0.01263	-0.00	1.000
Ferric ammonium citrate	0.00000	0.00000	0.01263	0.00	1.000
EDTA	-0.00000	-0.00000	0.01263	-0.00	1.000
Na_2Co_3	-0.00000	-0.00000	0.01263	-0.00	1.000

Data in table 2 indicate that all the media components were showed nonsignificance for the Exopolysaccharides (EXP) production (P – value > 0 . 5).These variables correlates negatively with it, highconcentration of these variables inhibits (EXP) production by *Aphanocapsa sp* except Ferric ammonium citrate correlates positively, highconcentration of this variable promote (EXP) productionby *Aphanocapsa sp*.The coeffient value for all variables is zero indicating independent variables.

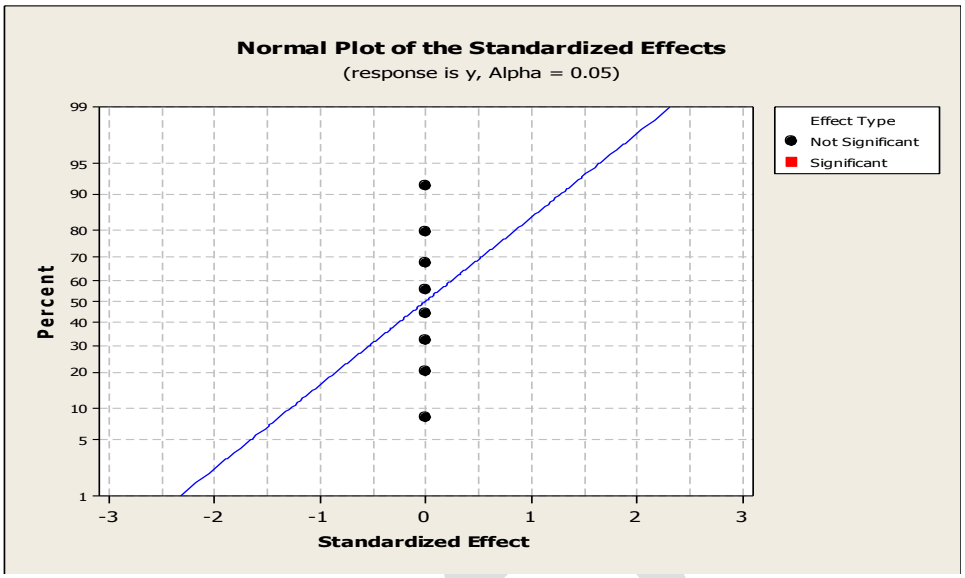

Fig.1.Normal plot of Estimated effects for analysis of Plackett–Burman design on (EXP) production.

The normal probability plot displays negative effects on the left side of the graph and positive effects on the right side of the graph. The above plot shows the independent variables on zero.

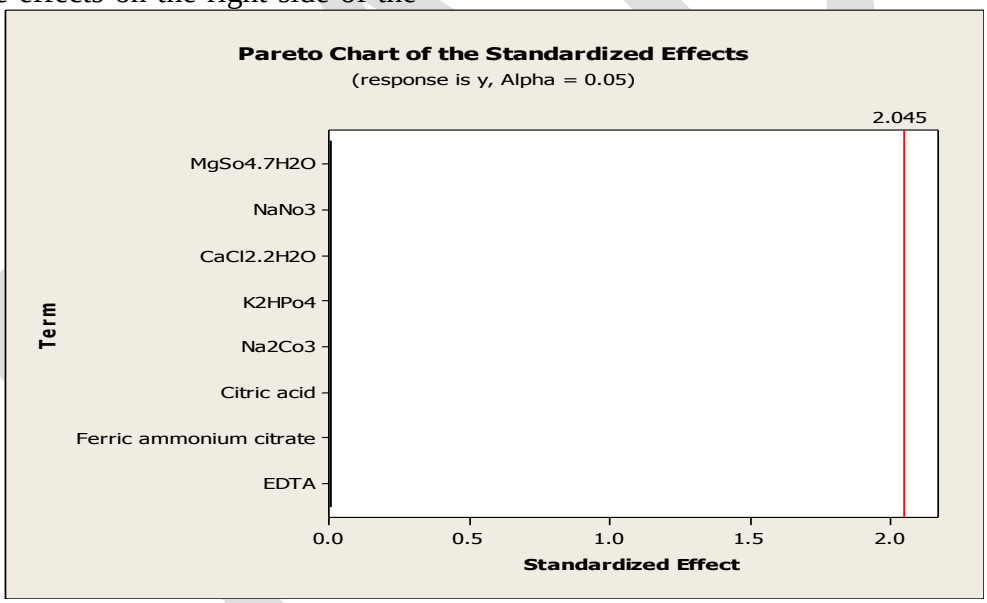

Fig.2 Pareto chart of estimated effects for analysis of Plackett–Burman design on (EXP) production.

Thepareto chart shows that there are no effect of the eight variables on the production of (EXP) by *Aphanocapsasp*this is due to the pareto charts are ranking the factors according to its effects and significance.

Table 3. Estimated effects and coefficients for analysis of Plackett–Burman design on antibacterial activity production.

Term	Effect	Coef	SE Coef	T	P
Constant		562.50	16.80	33.48	0.000
$NaNo_3$	105.00	52.50	16.80	3.12	0.004
K_2HPo_4	-45.00	-22.50	16.80	-1.34	0.191
$MgSo_4.7H_2O$	91.67	45.83	16.80	2.73	0.011
$CaCl_2.2H_2O$	48.33	24.17	16.80	1.44	0.161
Citric acid	-56.67	-28.33	16.80	-1.69	0.102
Ferric ammonium citrate	-53.33	-26.67	16.80	-1.59	0.123
EDTA	-81.67	-46.67	16.80	-2.78	0.010
Na_2Co_3	-93.33	-40.83	16.80	-2.43	0.022

The data presented in table 3 clearly show that p-values indicates that $NaNo_3$,EDTA, $MgSo_4.7H_2O$ and Na_2Co_3 are significant variables, Citric acid, Ferric ammonium citrate, $CaCl_2.2H_2O$ and K_2HPo_4 are nonsignificant variables. Furthermore, the positive correlation obtained by $NaNo_3$,$MgSo_4.7H_2O$, $CaCl_2.2H_2O$ and a negative correlation for K_2HPo_4,Citric acid,Ferric ammonium citrate, EDTA and Na_2Co_3.**Yin *et al.* (1997)** stated that, changes in phosphate, nitrate, calcium, irradiance and temperature all caused quantitative, but not qualitative, changes in toxin composition produced by the cyanobacterium *Lyngbyawollei*

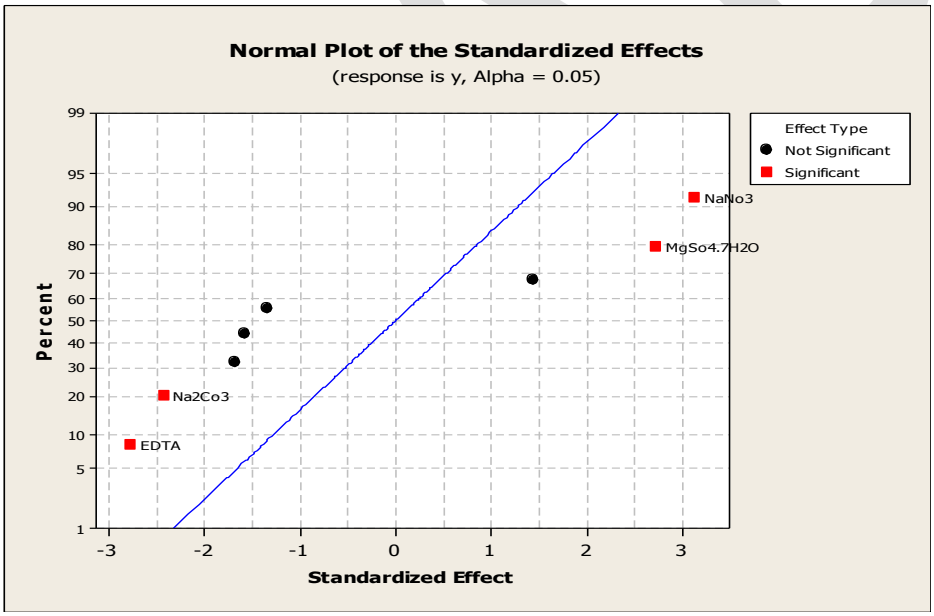

Fig.3.Normal plot of estimated effects for analysis of design on antibacterial activity.

By applying normal plot show the variableswas significant (had antibacterial activity against ***Streptococcus pyogenes*** (ATCC 19615)with positive correlation, $NaNo_3$ and $MgSo_4.7H_2O$ andsignificant with negative correlation, EDTA and Na_2Co_3, nonsignificant with positive correlation, $CaCl_2.2H_2O$ and nonsignificant with negative correlation, K_2HPo_4,Citric acid and Ferric ammonium citrate. Ohta*et al.*, (1995) observed that the increase in magnesium concentrations cause increase in antibiotic production from *Chlorococcun*strain HS-101..

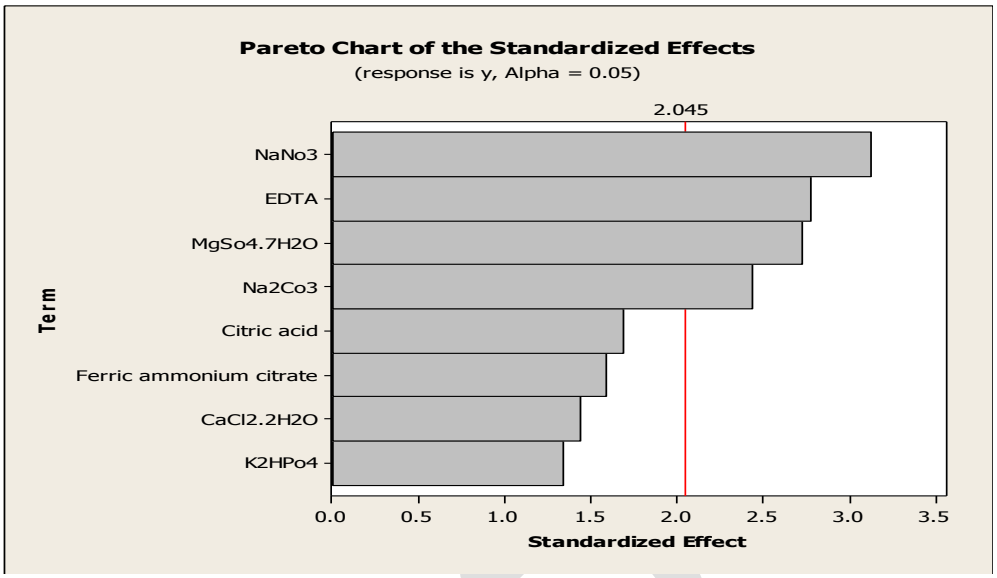

Fig.4 .Pareto chart of estimated effects for analysis of Plackett–Burman design on bioactivity of EPS against *Streptococcus pyogenes*(ATCC 19615).

Pareto chartrefers to $NaNo_3$ is the most effective factor versus to $K_2HPo_4$with the least effect on polysaccharide as antibacterial activity. This results agree with Bloor and England (1991) the increasing of nitrate from its base level of 8.8mM to26.4 mM increased the antibiotic production by *Nostocmuscorum.*

Table 6. Estimated effects and coefficients for analysis of Plackett–Burman design onExopolysaccharide productionof *Aphanocapsa sp*.

Term	Effect	Coef	SE Coef	T	P
Constant		7.568	0.3313	22.85	0.000
Ammonium Chloride	-0.182	-0.091	0.3313	-0.27	0.786
k_2HPo_4	3.018	1.509	0.3313	4.56	0.000
$MgSo_4.7H_2O$	0.965	0.482	0.3313	1.46	0.156
$CaCl_2.2H_2O$	0.602	0.301	0.3313	0.91	0.371
Citric acid	0.348	0.174	0.3313	0.53	0.603
Ferric ammonium citrate	-1.052	-0.526	0.3313	-1.59	0.123
EDTA	1.615	0.808	0.3313	2.44	0.021
Na_2Co_3	1.502	0.751	0.3313	2.27	0.031

The data obtained in table-6 shows the probabilities (p- value) that measures the evidence against the null hypothesis,the lower p- value (EDTA and Na_2Co_3) provide stronger evidence against the null hypothesis or significance.Whereas, the higher p- value (Ferric ammonium citrate, $MgSo_4.7H_2O$, $CaCl_2.2H_2O$, Citric acid and Ammonium chloride) indicate insignificance effect on the Exopolysaccharide production of *Aphanocapsa sp.*

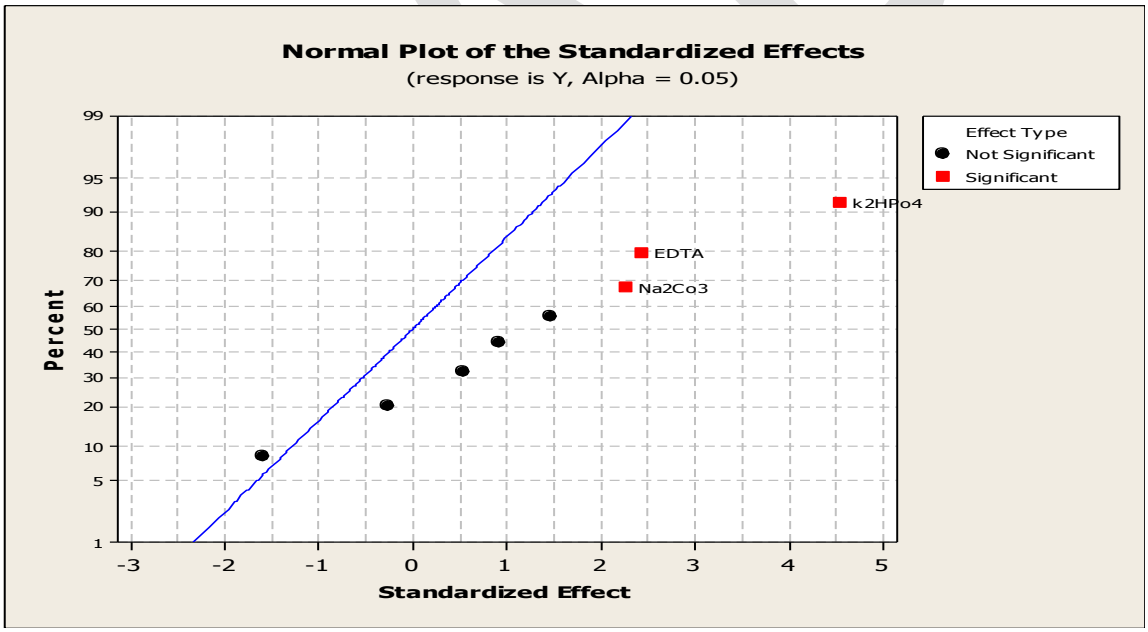

Fig.5.Normal plot of Estimated effects for analysis of Plackett–Burman design onexopolysaccharide production of *Aphanocapsa sp*.

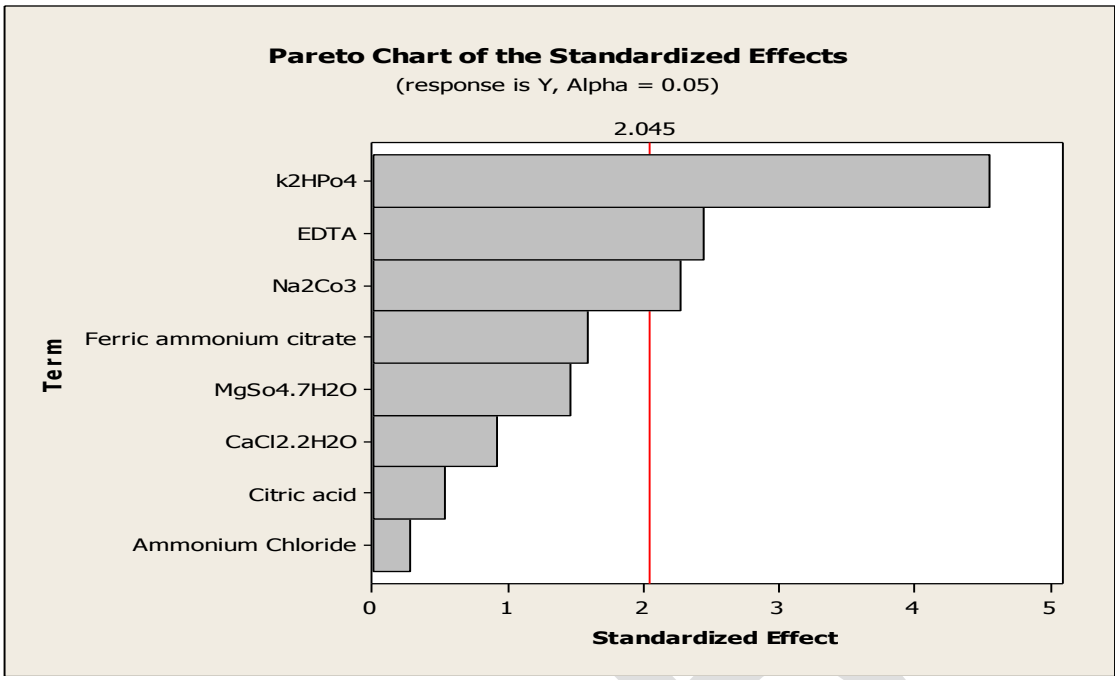

Fig.6 .Pareto chart of Estimated effects for analysis of Plackett–Burman design on Biomass of *Aphanocapsa sp*

The pareto chart shows that Na_2Co3 has the largest effect on exopolysaccharide formation while Ammonium chloride has the smallest effect, Effects are closely related to means, The effect is the mean for that level minus the overall mean for the factor.

Table 7. Estimated effects and coefficients for analysis of Plackett–Burman design on EXP that antibacterial activity against *Streptococcus pyogenes*(ATCC 19615)

Term	Effect	Coef	SE Coef	T	P
Constant		1.858	0.09258	20.07	0.000
Ammonium Chloride	-2.383	-1.192	0.09258	-12.87	0.000
k_2HPo_4	0.717	0.358	0.09258	3.87	0.001
$MgSo_4.7H2O$	-1.061	-0.531	0.09258	-5.73	0.000
$CaCl_2.2H_2O$	1.939	0.969	0.09258	10.47	0.000
Citric acid	-0.717	-0.358	0.09258	-3.87	0.001
Ferric ammonium citrate	1.050	0.525	0.09258	5.67	0.000
EDTA	0.717	0.358	0.09258	3.87	0.001
Na_2Co_3	2.394	1.197	0.09258	12.93	0.000

All the factors were proved to be significant onactivity of *Aphanocapsa sp*. Exopolysaccharide.The increase in K_2HPo_4, $CaCl_2.2H_2O$, Ferric ammonium citrate, EDTA and Na_2Co_3 corresponds to increase in exopolysaccharideactivity against ***Streptococcus pyogenes***, while the decrease of Ammonium chloride, $MgSo_4.7H_2O$ and Citric acid acompanied by increase of exopolysaccharide activity.

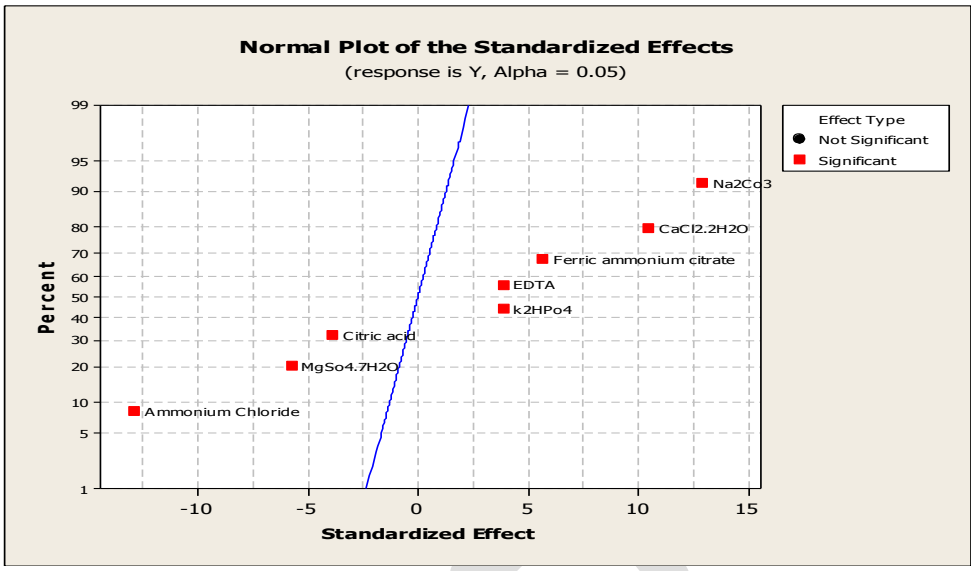

Fig.7.Normal plot of Estimated effects for analysis of Plackett–Burman design onbioactivity of (EPs) extracted from *Aphanocapsa sp* against *Streptococcus pyogenes*(ATCC 19615).

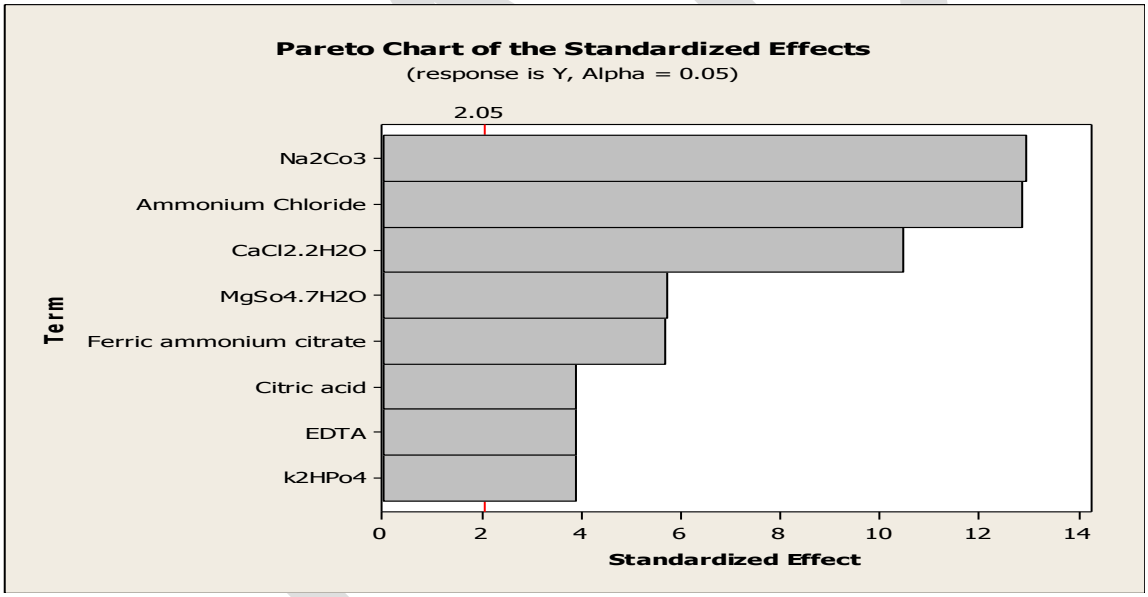

Fig.8 Pareto chart of Estimated effects for analysis of Plackett–Burman design on Exopolysaccharide activityagainst *Streptococcus pyogenes*(ATCC 19615).

Pareto chart ranks the detects from the largest to the smallest (Fig 8). Na_2Co_3 is the most effective factor on Exopolysaccharide activity **against*Streptococcus pyogenes***

(ATCC 19615) versus to K_2HPo_4.Exopolysaccharidesof*L. subnudus*is a bioactive secondary metabolite that possesses antibacterial properties which can be explored in the treatment of bacterial infections (Majolagbe et al.,2013)

CONCLUSION:

The results of these study revealed that Eexopolysaccharide extracted from*Aphanocapsa sp* had activity against ***Streptococcus pyogenes***(ATCC 19615).

BG11 medium supplemented with Ammonium Chloride shows a better results for Exopolysaccharide production from

Aphanocapsa sp than medium supplemented with Sodium nitrate .Exopolysaccharide production by *Aphanocapsasp* had antibacterial activity, and Plackett-Burman design proved to be effective in detecting which variables are more significant. **REFERENCES**

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