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Antimicrobial Effect of *SimaroubaGlauca*(LakshmiTaru) on Opportunistic Pathogens in HIV/AIDS patients

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Abstract: *Severalplant extracts exhibit antimicrobial activity against microbial pathogens. This suggests that plants which manifest relatively high levels of antimicrobial action may be the source of compounds that can be used to inhibit the growth of human pathogen[4] One of the ethnomedical plants Simaroubaglauca was investigated for Gas Chromatography Mass Spectrometry (GCMS) analysis to determine the chemical constituents present in different extracts of its leaves. The powdered leaf was subjected to successive extraction with five organic solvents. Out of 88 compounds reported most of the compounds were found to be medicinally effective, which could be useful against opportunistic infections in patients with poor immunity. The current work focuses on the extraction and assay of antimicrobial components from the leaf of Simaroubaglauca and also to evaluate their antioxidentalpotential, which is probably a new alternative for arresting the opportunistic infections in HIV/ AIDS patients thus extend their life span. The clinical samples collected from HIV/AIDS patients were used to isolate the most commonly found opportunistic infections causing pathogens namely Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa and Candida albicans. Extracts from leaves of Simaroubaglauca were tested against the above mentioned organisms and they were found to be effective. In addition to all phytochemical compounds needed farther investigations for toxicological aspects leading to therapeutic uses.*

Keywords:*Simaroubaglauca, GCMS, Opportunistic infections, Staphylococcusaureus, Streptococcus pyogenes, Pseudomonas aeruginosa, Candidaalbicans,*

I. Introduction

*Simaroubaglauca*has a long history of use in herbal medicines in many countries. *Simaroubaglauca*is one of the important herbal drugs used against dysentery hence its bark is also known as dysentery bark. The bark and leaf extract of Simarouba is well known for its different types of pharmacological properties such as haemostatic, antihelmenthic, antiparasitic, antidysentric, antipyretic and anti-cancerous. A rainfed wasteland evergreen edible oil tree, *Simaroubaglauca*, is commonly known as 'Laxmitaru' or 'paradise tree' belong to the family *Simaroubac*eae. The specific name glauca means covered with bloom which refers to the bluish green foliage [1]. The leaf, fruit, pulp and seed of *S. Glauca*are known to possess medicinal properties such as analgesic, antimicrobial, antiviral, astringent,emmenagogue, stomachic, tonic and vermifuge. Studies also revealed that it has strong inhibitory activity against protozoa. However, till date not many reports are available about its use its antifungal properties [2]. The present study is carried out to find the antibacterial and antifungal effect on of the leaf extract of *Simaroubaglauca*on the opportunistic infectionscausing agents most commonly found inHIV/AIDS patients suchas *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa and Candida albicans.*

II. Material and methods

2.1. Collection of Plant materials:Fresh leaves were collected and dried in the room temperature. Fine powdered leaves were loaded in Soxhlet extraction apparatus and extraction was done with Ethanol and distilled water. Forty gram of plant material was subjected to 175 ml Ethanol at 65 C temperature and extraction was carried out for 5 hours. The colour of the extract was dark green.The same amount of plant material was subjected to extraction with distilled water at 100 C temperature for 5 hours. The extract was in dark brown in colour. Ethanolic extract was air dried, weighed and stored for future use. Distilled water extraction was measured and stored in collection bottles.

2.2. Preliminary qualitative Phytochemical Screening: Initially, the extracts were subjected to qualitative and quantitative analysis for various phytochemical constituents including alkaloids, carbohydrates, steroids, proteins, phenols, tannins, flavonoids, glycosides, gums, saponins and terpenes.[6]

2.2.1. Detection of Phenolic flavonoids: One ml of filtrate with 2 ml of 10% lead acetate gives brown precipitate indicating the presence of Phenolic flavonoids.
2.2.2. Detection of Flavonoids: One ml of filtrate with 2 ml of dilute NaOH shows development of golden yellow colour indicating the presence of Flavonoids.
2.2.3.. Detection of Carbohydrates: One ml of the filtrate with 5ml of Benedict's reagent was boiled for 5 minutes. Bluish green colour indicates the presence of carbohydrates.
2.2.4. Detection of Proteins and Amino Acids: Hundred mg extract was dissolved in 10 mL of distilled water and filtered through Whatmann No.1 filter paper. Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) was added to 2 mL of aqueous filtrate. A characteristic blue colour appearance indicates the presence of amino acids and whereas the appearance of purple colour indicates the presence of protein.
2.2.5. Detection of Anthocyanides: One ml of filtrate with 5ml of dilute HCl shows the presence of pale pink colour indicating the presence of Anthocyanides
2.2.6. Detection of Anthraquinones : About fifty mg of methanolic extract was heated with 10% (w/v) ferric chloride solution and one mL of concentrated hydrochloric acid. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. The formation of pink or deep red coloration of aqueous layer indicates the presence of anthraquinones.
2.2.7.Detection of Saponins: One ml of filtrate with 2ml distilled water, shake vigorously allow it to stand for 10 minutes. Development of foam on the surface of the mixture, lasting for 10 minutes indicates the presence of saponins.
2.2.8 Detection of Phlobatannins: To one ml of the filtrate add a few drops of 1% aqueous HCl. A red precipitate is formed indicates the presence of Phlobatannins.
2.2.9.Detection of Tannins: One ml of filtrate with 2 ml of Ferric chloride gives dark green colour indicating the presence of Tannins.

2.3.GCMSanalysis:The crude extracts were added to GC-MS analyses and were found to contain a high number of metabolites. The Gas chromatography (GC) analysis was carried out on a 7890A.[4] GC chromatograph fitted with HP-5 MS column (30 m x 0.25 mm, 0.25 μm) and interfaced with a mass spectrometer 5975C. The GC analytical conditions were as follows: carrier gas He (99.999% purity; 1 mL/min), injector temperature 280°C, column temperature programmed from 100 °C (4 min hold) to 300 °C (16 min hold) at 10°C/min. The samples were injected by splitless mode.[5] The volume injected and the inlet pressure were 1.0 μL and 72.553 kPa, respectively; and the total running time was 36 minutes.

2.4. Microorganisms used: The samples were collected from the clinical samples of the HIV/AIDS patients. Standard identification of the opportunistic infection causing four agents were done and used to check inhibitory effect of the herbal extracts on them by using Spectrophotometry.

2.5. Antimicrobial activity:Serial dilution techniques are routinely used in hospitals, public health, virology, immunology, microbiology, pharmaceutical industry, and food protection [7]). A serial dilution is a series of sequential dilutions used to reduce the chemicals present in the crude extract (stock) to different concentration. Each dilution will reduce the concentration of the chemicals by a specific amount. **Steps involved –** 1. Prepare six test tubes with nine ml of dilution liquid. These tubes will serve as the dilution blanks. Then One ml of the extract was added to the test tube containing nine ml of nutrient broth. Then diluted sample to the first tube and then serially diluting into the following tubes. 2. Draw 1 mL of undiluted solution from test tube with a pipette and transfer it to the test tube labelled 1:10 containing nine mL of the dilution liquid and mix thoroughly. There is now 1mL of the undiluted solution in 9 mL of the dilution liquid. The solution, therefore, has been diluted by a factor of 10. 3. For the second serial dilution, we will take 1 mL of solution from tube 1:10 and add it to the 9 mL of dilution liquid in the tube 1:100. Thoroughly mix tube 1:10 before adding to the next tube. Again, mix the tube 1:100 following dilution. The solution from test tube 1:10 has been diluted 10-fold into test tube 1:100.4.This process may be repeated as many times as necessary to achieve the desired solution(1:10-1:10,00000=10-1ml-10-6ml).The nutrient broth used as positive control and the negative control the respective solvents.

2.6. A spectrophotometer is an instrument for measuring the absorbance of a solution.

Absorbance is a useful quantity. The Beer-Lambert law states that: $A = \varepsilon cl$where A is the absorbance of the sample at a particular wavelength, is the extinction coefficient for the compound at that wavelength in (M•cm)-1, ***c*** is the molar concentration of the absorbing species, and ***l*** is the path length of the solution in cm. Thus, if the extinction coefficient of an absorbing species is known, the absorbance of the solution can be used to calculate the concentration of the absorbing species in solution. (This assumes that the species of interest is the only material that absorbs at the wavelength being measured.UV spectrophotometer was used to check absorbance at 650nm. The rate of absorbance allows us to calculate the concentration of compounds/ number of cells in solution [9].50μl of the overnight cultures of the species of *Pseudomonas,Candida, Streptococcus, and Staphylococcus* are inoculated into the different concentrations of the extract in 2ml Ependoff vials and are incubated at 37˚C. They are then diluted with 2.5ml of water each before taking their optical density values in the spectrophotometer. The Optical density (OD) values are taken at different intervals of time based on the doubling time of the microbes after incubation. The broth used for making the overnight cultures of the organisms is maintained as the control throughout the experiment while measuring the optical density values till the last set of OD value of the samples have been recorded. When the rate of absorbance decreases indicates the decrease in the number of cells present.

III. Results

3.1.. Phytochemical screening showed the presence of Phenolic flavonoids, phenols, flavonoids, carbohydrates, proteins, aminoacids, Tannins, phtotanins, Anthocyanides , Anthroquinones and saponins .

3.2.GCMS:Gas Chromatography Mass Spectrometry (GCMS) is one of the techniques used to identify the phytochemical compounds present in the plant materials. The graph of the GCMS analysis shows the different peaks of the various compounds present in it (Fig 1 and Fig 2).GCMS chromatogram in figure 1 shows 19 compounds in various peaks.(Table 1) Among them 2-Furancarboxaldehyde, 5-(hydroxymethyl)- shows on maximum peak.(Fig 1) Periodic measuring of the absorbance of the microbes introduced with plant extracts in different concentrations gave promising results.(Fig 3 to Fig 10). The rate of absorbance is directly proportional to the number of cells present. These serially diluted extracts were used for the experiments. Antibacterial activity of three different concentrations of extracts of *S. glauca*have been evaluated *in vitro* against gram positive and negative bacteria that are known to cause infections in human pathogens.[11]

Table 1: Phytochemical Constituents Present in the leaf of*Simaroubaglauca*

Compound	Petroleum ether	Acetone	Ethyl acetate	Ethanol	Aqueous
1. Phenolic flavonoids	+	+	+	+	+
2.Phenols	+	+	+	+	+
3.Flavanoids	+	–	+	+	+
4.Carbohydrate	+	+	+	+	+
5. Protein	–	–	–	–	–
6. Amino acids	–	–	+	–	–
7. Anthocyanides	–	–	–	–	–
8. Anthroquinones	+	+	+	+	+
9.Saponins	+	+	+	+	+
10. Phlobatannins	--	–	–	–	+
11. Tannins	+	+	+	+	+

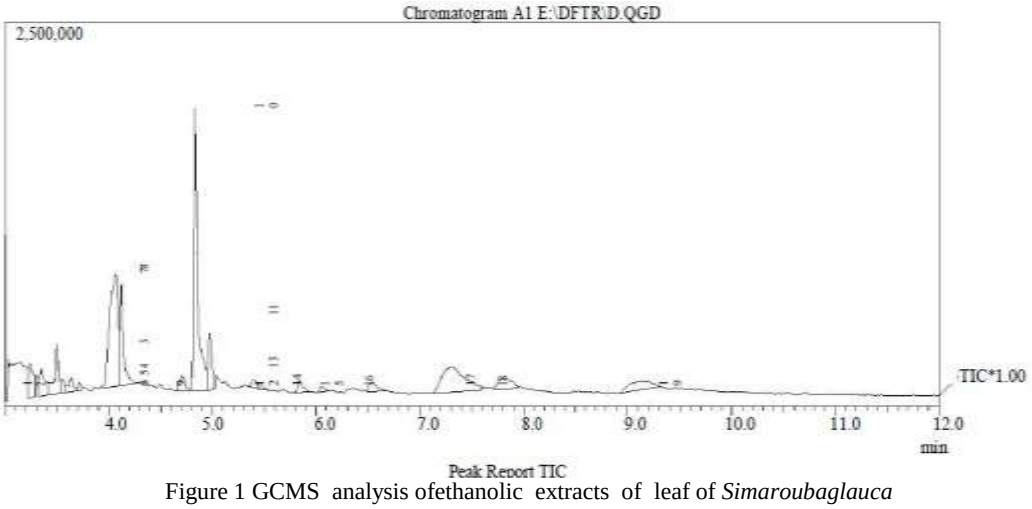
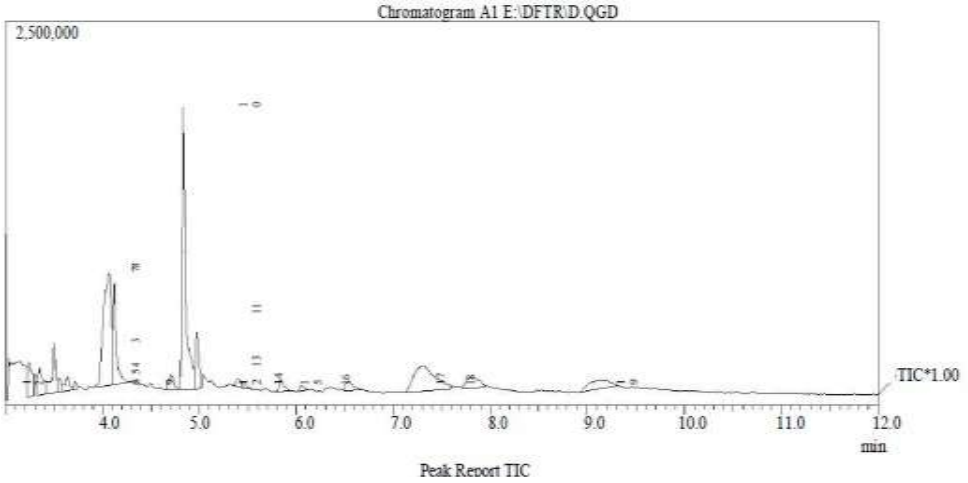


Figure 1 GCMS analysis ofethanolic extracts of leaf of *Simaroubaglauca*

Table 2 GCMS analysis ofethanolic extract of leaf *of Simaroubaglauca*

	Compound	Biological activity	Chemical formula	Molecular weight (mg/ml)	Retention time	Area	Area%
1.	1,3-Dioxol-2-one, 4,5-dimethyl	Antibacterial [10]	$C_5H_6O_3$	114.09934	3.239	800113	4.04%
2.	Di-n-octyl phthalate		$C_{24}H_{38}O_4$	390.55612	3.348	656032	3.31%
3.	2-Propenoic acid,		CH_2CHCO-	100.11582	3.550	219039	1.11%
4.	Ethyl ester (CAS) Ethyl acrylate		COC_2H_5				
5.	Heptacosane	Antimicrobial and antioxidant [14]	$C_{27}H_{56}$	380.73354	3.635	307270	1.55%
6.	Butane, 2-methyl- (CAS) Isopentane	As anti anesthetic[15].	C_5H_{12}	72.14878	3.721	106844	0.54%
6.	Propanal, 2,3-dihydroxy-	Antimicrobial agent [16]	C3H6O3	90.07794	4.068	4162984	21.03%
7.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	Antimicrobial Anti-inflammatory [16]	$C_6H_8O_4$	144.12532	4.122	1536834	7.76%
8.	Furancarboxaldehyde, 5-hydroxymethyl)-	Antimicrobial [16]	$C_6H_6O_3$	126.11004	4.834	748334	23.99%
9.	1,2,3-Propanetriol, Monoacetate	Antimicrobial [16]	$C_5H_{10}O_4$	100.11582	4.975	905014	4.57%
10.	Furancarboxaldehyde, 5-(hydroxymethyl)-	Antimicrobial[16]	$C_6H_6O_3$	126.11004	4.834	748334	23.99%
11.	4H-Pyran-4-one, 2,3-	DNA breaking activity	$C_6H_8O_4$	144.12532	5.395	187132	7.76%
12.	dihydro-3,5-dihydroxy-6-methyl-	Antioxidant and mutagenicity[16]					
13.	2-Butanone, 4-hydroxy-3-methyl-	Antioxidant, Antimicrobial[16]	$C_5H_{10}O_2$	102.1317	5.467	106510	0.54%
14.	Octadecanal	Decarbonylase activity activity[16]	$C_{18}H_{36}O$	268.47784	5.838	281797	1.42%
15.	Tetratetracontane	Antifungal[16]	$C_{44}H_{90}$	619.1854	7.803	663028	3.35%

16.Vitamin E	Antidermatitic, Antileukemic, Antitumor, Anticancer, Hepatoprotective, Antispasmodic [16]	$C_{29}H_{50}O_2$	430.	6.537	353236	1.78%



Figures 2.GCMS analysis of the aqueous extract ofthe leaf of *Simaroubaglauca*

Table 3 showing the GCMS analysis of Aqueous extract of the leaf of*Simaroubaglauca*

Sl number (	Compound	Biological function	Chemical formula	Molecular weight mg/mol)	Retention time	Area	Area%
1.	Propanoic acid	Antibacterial Antifungal[16]	CH_3CH_2COOH	74.07854	4.180	12384487	12.55%
2.	1-Dodecene		$C_{12}H_{24}$	168.31896	4.224	752951	2.79%
3.	1-Tetradene	Determination of phosphatase activity [16]	$C_{14}H_{28}$	196.37212	4.941	34465898	34.92%
4.	2-Furancarbox-aldehyde, 5--(hydroxy- methyl)-	Antibacterial [16]	$C_6H_6O_3$	126.11004	5.074	25525501	25.86%
5.	1,2,3-Propanetriol, monoacetate	Antibacterial [16]	$C_5H_{10}O_4$	134.1305	5.208	4679433	4.74%
6.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl--(CAS) 3 ,5-DIHY	Antibacterial [16]	$C_6H_8O_4$	144.12532	5.816	663700	1.69%
7.	Neophytadiene	Antipyretic, analgesic, and anti-inflammatory, Antimicrobial, antioxidant [16]	C_20H_{38}	278.51572	6.081	471079	1.49%

8.	1,3-Dihydroxy-4-hexene	Antimicrobial, Antioxidant [16]	$C_{11}H_{21}NO_4$	231.28874	6.892	2722754	2.76%
9.	Benzoic acid, 4-hydroxy-, methyl ester (CAS) Methyl p-hydroxybenzoa	Antiseptic agent Disinfectant [16]	$C_{16}H_{24}O_3$	152.149	7.356	2592949	2.63%
10.	Tridecanol (CAS)	Antibacterial[16]	$CH_3(CH_2)_{11}CH_2OH$	200.36082	7.350	2592949	3.79%
11.	Octadecanal	Catalase activity [16]	$C_{18}H_{36}O$	268.47784	9.300	1432972	1.45%
12.	Tetradecanal		$C_{14}H_{28}O$	212.37162	12.776	509186	0.52%

Graphs showing the rate of absorbance which is directly proportional to the growth rate

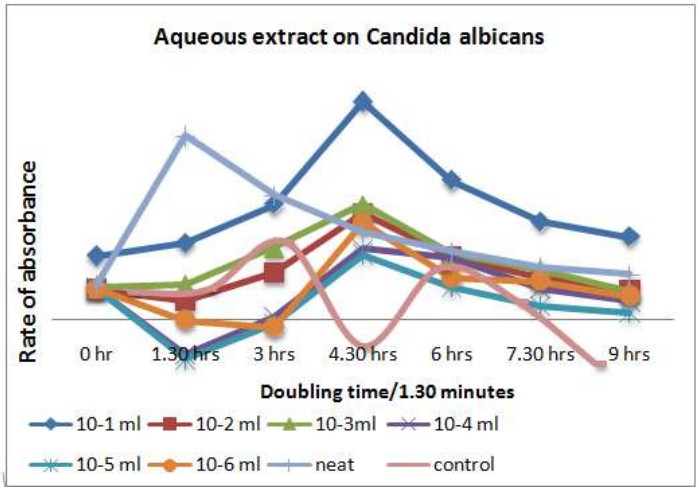
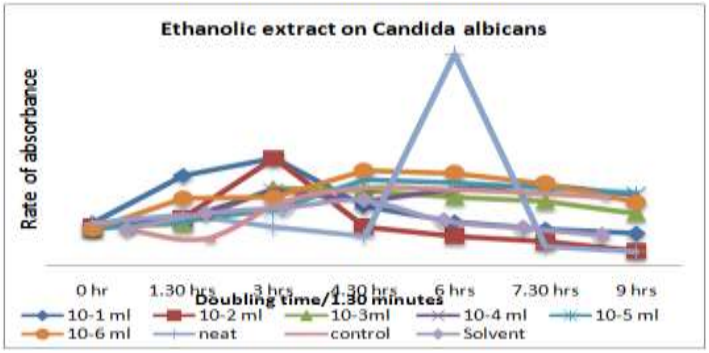


Figure 3



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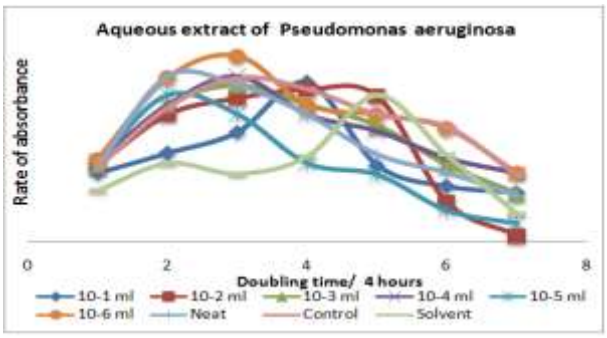

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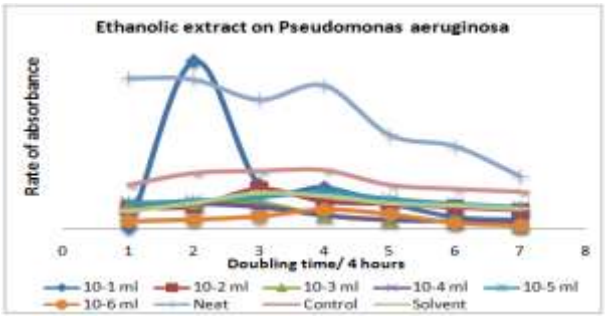

Figure 6

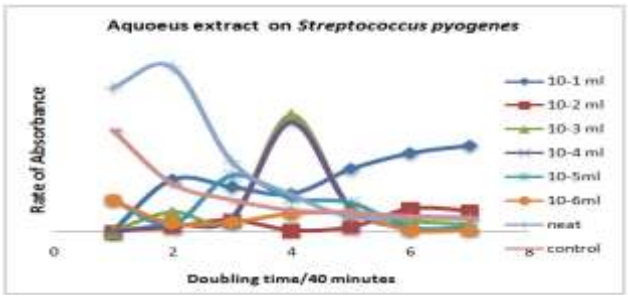

Figure 7

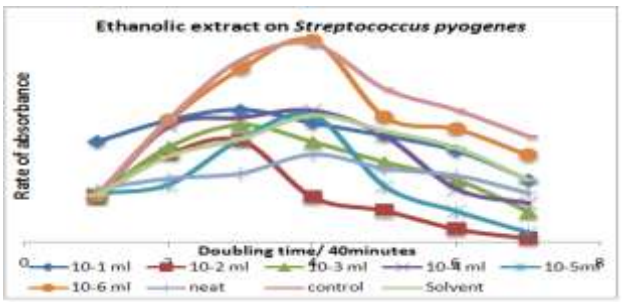

Figure 8

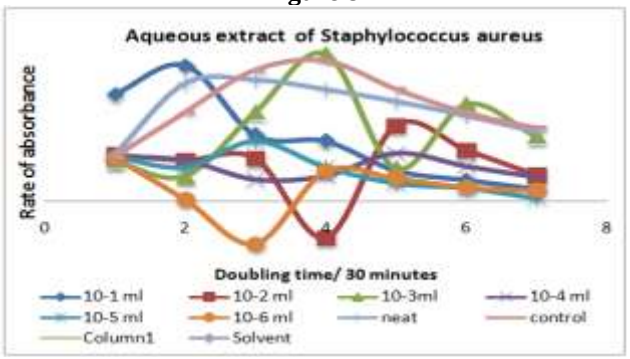

Figure 9

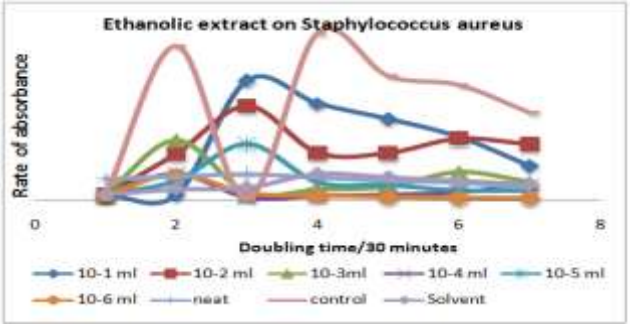

Figure 10

IV. Conclusion

The leaf of*Simaroubaglaouca* are found with a total of 88 compounds, identified from the five extracts of (38 from petroleum ether, 20 from ethyl acetate, 6 from Acetone, 19 from Ethanol and 13 from Aqueous). A few are being common to some of the extracts), and the most abundant being ethyl ester (CAS) 1-Dodecene (44.91%), Ethyl propanoate (41.09%), 1-Tetradene (34.92 %),2-Furancarboxaldehyde, 1-Tetradecene (27.43%), 5-(hydroxymethyl) (25.86%),2-Furancarboxaldehyde, 5-(hydroxymethyl)-(23.99%), 2-2-Octadecen-1-ol (21.21%), propanal,2,3-dihydroxy-(21.03%), Propanoic acid, NEOPHYTADIENE(18.41%), Vitamin E (14.31%) and Octadecanal (CAS) Stearaldehyde (12.58 %).All five extracts were tried on all four microbes and they all showed inhibitory effect due to the presence of bioactive compounds which are antioxidant or antimicrobial properties. Many plants used in traditional medicine worldwide contain saponins, which can often account for their therapeutic action like antibacterial, antiviral, anti-inflammatory, antiprotozoal and antitumor activities[11].The introduction of the serially diluted nutrient liquid with plant extract was used with 50 microlitre (µl) of the inoculum for the periodic checking of the rate of absorbance of the cells which showed promising results. It proves that the extracts of*Simaroubaglaouca*whichcould be used as an alternative to bring down the opportunistic infections rate in patients with poor immunity very specially for people with poor immunity such as HIV/AIDS patients.The rapid propagation in antibiotic resistance and the increasing interest in natural products have placed medicinal plants in the front lights as a reliable source for the discovery of active anti-microbial agents and possibly even novel classes of antibiotics (Schultes RE, 1960, 2011).The promising inhibitory effect of *Simaroubaglaouca on* Opportunistic infection causing agents could be an alternative for arresting infections in HIV/AIDS.Thus increase their life span.

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