

Medical Potential Of A Locally Sourced Lipids Mixture On Confirmed Corona Virus Infected Patients And Those At Risk of Infection, Attending National Hospital Abuja, Nigeria.-An Evidence Based Approach

Ajobiewe OJ^{1,2*}, Ajobiewe HF³, Ogundeji OA², Umeji LC², Umeji NC⁴, Olagolden S⁵, Ajobiewe DC⁵

¹National Hospital Abuja, Plot 132 Garki Central District, Nigeria

²Imo state University Owerri Nigeria

³Bingham University Km. 26 PMB 005, Abuja -Keffi Expressway Karu, Nasarawa State National Hospital Abuja Plot 132 Garki Central District, Nigeria

⁴Chemical Pathology Department, Federal Medical Centre, Jabi Abuja Nigeria

⁵University of Abuja Nigeria

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*Corresponding author: Ajobiewe OJ

Abstract

Original Research Article

Forty volunteers (comprising of four families whose members were both amalgamation of nuclear and extended) and eight COVID 19 disease confirmed positive cases unisex attending National Hospital Abuja either as in /out patients randomly participated in this pilot study; The sole objective was to determine the effectiveness of Joseleen herbal lipids mixture as both therapeutic and prophylactic agent against COVID 19 disease. We initially proposed Joseleen Herbal Mixture as a prophylactic agent for COVID19 disease, following its high potential of protecting individuals against Coronavirus infection, as evidenced from haemagglutination inhibition assay^{16,17} carried out using influenza virus, a closely related virus with 80% sequence homology. Randomized assay conducted on those infected with COVID 19 disease revealed its high therapeutic potential, after administering informed consent questionnaires to volunteers. Four indicators were used to critically appraise the herbal lipids mixture. These were, Percentage overall rating of the herbal lipids Mixture by volunteers; Their prophylactic experience with the herbal mixture after staying with COVID 19 patients for a period between 20 – 60 days; Curative experience of the volunteers who were infected initially with corona virus; Side effect experience of these same group of volunteers. The outcomes showed, percentage ratings of 83.1%, 94.43%, 100% and 17% respectively. ANOVA statistic technique revealed significantly different high percentage ratings among the first three indicators ($P \leq 0.05$). However, the side effect percentage rating was significantly lower than the other three indicators ($P \leq 0.05$). Thus, Joseleen herbal mixture is both effectively therapeutic and prophylactic against COVID 19 pandemic disease. The case fatality rate was zero % with respect to this research, using Joseleen herbal lipids mixture as COVID 19 remedy.

Keywords: Corona Virus Disease 2019 (COVID 19), Joseleen Herbal Mixture, Indicators, Percentage ratings, Therapeutic, Prophylactic.

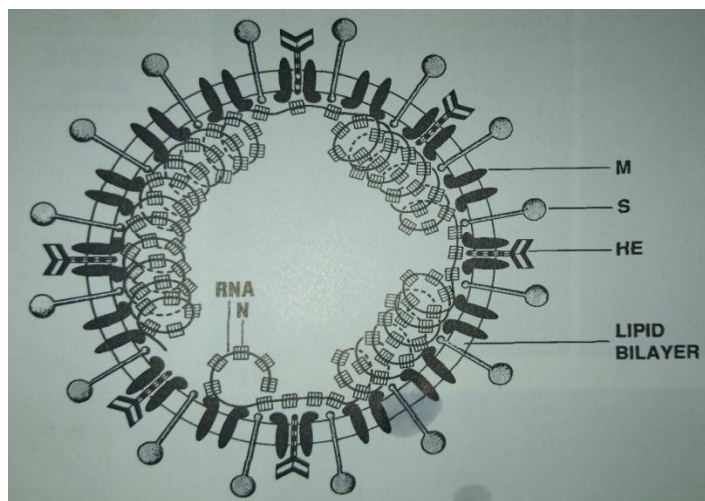
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INTRODUCTION

The locally sourced lipid mixture comprises of very important semisolid medicinal oils that have been found very useful in the past for effectively curing viral infections. These originated from plants like *Aloe vera*, *Vitellaria paradoxa*, *Elaeis guineensis* and *Melaleuca alternifolia*. This goes to further stress the importance of biodiversity in providing buffer and lasting solution to most of our environmentally generated problems. Essentially this mixture has natural Saponin, other very

important phytochemicals, fatty acids such as oleic acid, stearic acid, arachidic acids and even medicinal furfural aldehydes constituents which are highly medicinal- being protein sequestering and anti-inflammatory agents respectively [1, 2]. The lipid base of corona virus readily solubilizes when it comes in contact with the lipid mixture termed Joseleen herbal mixture in the light of the above reasons and from basic chemistry principle of 'like dissolves like' [3].

Structure of Coronavirus



Courtesy: Fields Bernard N: Fundamental Virology 3rd Edition 1995.

M is intermembrane protein (Mr180000-220,000)

S is surface glycoprotein (Mr27000-350000)

He is Hemagglutinin esterase (Mr 65000)

N is the Nucleocapsid (Mr 19000-60000)

RNA is the Genomic Ribonucleic Acid which serves as mRNA for RNA polymerase [4]

Corona viruses belong to the family of Coronaviridae. The family has two antigenic groups domiciliated in both human and animals' hosts. The first antigenic group (Antigenic group I), the virus type, and the hosts are respectively stated as follows; Human respiratory Corona virus, HCV 229, Porcine transmissible gastroenteritis virus, PTGEV, Canine corona virus, CCV, Feline enteric corona virus, FECV, Rabbit corona virus, RBCV, their hosts are humans, pigs, dogs, Cats, and Rabbits. In humans, pigs and cats;⁵ the disease they mainly manifest is respiratory; while in rabbits it is immunological disorder, infectious peritonitis, nephritis and pancreatitis. In dogs, they cause enteric infection while also in some cats, they cause hepatitis, neurologic diseases, e.t.c. In the second antigenic group (Antigenic group II), the virus type, and the hosts are also respectively stated as follows; Human respiratory Corona virus, OC43, Porcine hemagglutinating encephalomyelitis virus HEV, mouse hepatitis virus MHV, Sialodacryo adnavirus

SDAV, Bovine corona virus, BCV, Rabbit corona virus, RBCV, and Turkey Corona virus [6]. Their hosts are humans, pigs, mice, rats, turkeys. In humans, the disease they mainly manifest is respiratory; while in pigs it is respiratory, enteric, and neurologic infections; in mice it is respiratory, enteric, hepatitis and neurologic infections in rats it is mainly neurologic disease

In cows and rabbits, they cause enteric infections while also in turkeys, they cause respiratory and enteric diseases [7].

Briefs on Coronavirus Attachment

Virions bind to the plasma membrane by interaction of the S proteins in the large spikes with specific receptor glycoproteins. Virions of the antigenic group II uses the HE glycoproteins for pre- receptor interaction with the 9-O acetylated neuramic acid residues on the plasma membrane Penetration occurs by S protein mediated fusion of the viral envelope with the plasma membrane or for some stains with endocytic membranes[8]. The genomic RNA is translated to form polyprotein (>800kd) which is co or post translationally processed to yield multiple protein that serve as a virus specific, RNA –dependent RNA polymerase. These may also serve other roles in viral transcription and replication processes. The RNA dependent RNA polymerase uses the positive genomic RNA strand as template for full length negative strand RNA which is replicated to form new positive strands genomic RNA. The genomic RNAs and mRNAs are capped and polyadenylated. At the 5¹ ends of each mRNA is a common leader sequence about 70 nucleotides long that is encoded by the 3¹ends of the negative strand template. The genome length and the sub genomic, negative strand RNAs each have a sequence complimentary to the leader sequence at the 3¹ends. With just few exceptions, each polycistronic mRNAs is translated to yield only the polypeptide encoded at the 5¹ ends of the mRNA. For instance, mRNA 6 encodes the M protein. The N protein and newly formed genomic RNA assemble in the cytoplasm to form helical nucleocapsid[9].

Characteristics of Corona Viruses

Coronaviruses are

- Virions are 80-220nm in diameter
- Pleomorphic but roughly spherical in shape.

The nucleocapsid is helical with diameter of 10-20nm Molecular weight- ratio is approximately 400 x10⁶ Buoyant density is 1.23-1.24 g/cm in CsCl

- Genome consist of a single molecule of linear positive sense, single stranded –RNA, 20-30 Kb in size. The RNA has a 5¹-terminus cap and a 3¹ –terminus poly(A) tract [10, 11]
- Virions contain a large surface glycoprotein, S, Mr180,000-220000
- Intermembrane protein, M, (Mr 27000-35000)
- Nucleocapsid protein, N, (Mr19000- 60000)
- Some corona viruses contain a Hemagglutinin esterase protein, HE, (Mr 65,000) that form short surface projections and a small membrane protein
- These form short surface projections and a small membrane protein, SM, (Mr, 10000 - 12000)
- Virions contain lipids in their envelop and some of their surface proteins are heavily glycosylated. These form short surface projections and a small membrane protein, SM, (Mr, 10000 -12000) [12, 13].
- Virions contain lipids in their envelop and some of their surface proteins are heavily glycosylated
- The genomic RNA serves as the mRNA for the RNA polymerases (Pol 1a Mr 440,000-500,000; Pol 1b, Mr300,000-308,000)
- When translated, the polymerase components are responsible for the formation of full length complementary and progeny RNA species
- They are also responsible for the production of sub genomic mRNAs
- One species of genome length complimentary RNA acts as a template for the synthesis of a 3¹ coterminal nested set of genomic mRNAs that are 5¹ capped and 3¹ polyadenylated [14, 15].
- Synthesis of mRNA species from this template involves a process of discontinuous transcription, perhaps due to leader priming mechanism.
- The virus synthesizes five to seven major sub genomic mRNA (this varies with the virus strain in question).
- Only the 5¹ –unique regions of the mRNAs are thought to be translationally active.
- Virions mature in the cytoplasm by budding through the endoplasmic reticulum and Golgi membranes
- The viruses have narrow host ranges[15]

The common routes of transmission are

- Aerosol
- Fecal Oral
- Fomite [15]

Physical Properties of Corona Viruses

Virions are sensitive/lethal to

- Heat
- Nonionic detergent
- Lipid Solvent
- Formaldehyde
- Alcohol
- Oxidizing agents[15]

METHODOLOGY

Forty volunteers (comprising of four families whose members were both amalgamation of nuclear and extended) and eight COVID 19 disease confirmed positive cases unisex attending National Hospital Abuja either as in /out patients randomly participated in this pilot study. The four families comprised of ten, seven, five, and ten members per family unisex. At least one member per family regularly interacted with COVID 19 infected patients in some health settings by virtue of their professions. All volunteers were in the age range of \geq ten years to \leq 60 years unisex. The four family members regularly used Joseleen herbal mixture for a period of \geq twenty days to \leq sixty days prophylactically. Following this, each of the four health workers from each family who had regular interactions with the COVID 19 patients underwent the RT PCR test for Corona virus infection alongside the other eight corona virus infected volunteers. This constituted the in-vivo assay we carried out. While the in vitro assay tested its haemagglutination inhibition potential on a closely related virus i.e. influenza virus. This assay was retrospectively carried out by digging into the former work earlier performed by our MPH students on MOUSE ADAPTED INFLUENZA “A” (A/PR/6/32) VIRUS since it has over 80% sequence homology with corona virus and that had already been published [16, 17].

In -vivo Assay followed this sequence

Inclusion criteria from the research work

- All health workers exposed by virtue of their job’s schedules or otherwise to infected COVID 19 Patients
- Any individual either Male or Female within the age bracket (\geq 10 years \leq 75years) exposed to COVID 19 through any means
- All those at risk due to recent travels, meetings, ceremonies, failure to obey lay down regulation for prevention of infection, attendance at funerals e.t.c.
- Already infected individuals whose histories reflect/imply A and B above
- Those without any major underlying ailments

Exclusion criteria from the research work

- Anybody that is immune suppressed due to underlying ailments or any life-threatening disease/s
- Infants or Children below the age of ten years
- Those seventy -five years and above
- Those that have mental health challenges
- Those that have general allergic reactions to lipids

General Questions for volunteers

If you have been using this herbal mixture on prophylactic basis in a high-risk environment, then kindly furnish us with the following information;

- A) For how long
- B) Any side effects
- C) In what location/environment?
- D) How close are you to those already infected in terms of job interactions and otherwise?

Comment Freely on This Herbal Mixture

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SECTION 2

Feedback from Client

How long did it take for you to notice /observe/record changes in symptoms?

- A) After 24 hrs. of herbal mixture administration
- B) After 36 hrs. of herbal mixture administration
- C) After 48 hrs. of herbal mixture administration
- D) After one week of herbal mixture administration
- E) After two weeks of herbal mixture administration
- F) Greater than two weeks of herbal mixture administration
- G) No symptoms observed

- H) Can't describe changes
- I) No changes observed

How would you rate the Joseleen herbal mixture?

- A) Excellent
- B) Very Good
- C) Good
- D) Average
- E) Poor
- F) Very Poor
- G) Not ratable

Questionnaires were randomly administered to a cohort of in/out patients, whose contents were as depicted and also conformed to the criteria defined above and below;

Biodata/Subject identifier; - viz;- Date, Age, Sex, Name, Title, Occupation, Country, Geopolitical Zone, State, Tribe, Religion, Nigerian Public Health Epidemiological Number (CODE), National Identification Number, Passport Number, Any other Genuine means of identification, How the participant in the project herd about us.

HYPOTHESES

H₀: No significant differences exist between the four indicators which are, the general overall Joseleen herbal mixture rating per in/outpatients who volunteered, prophylactic experience of volunteers, curative experience, and side effects of Joseleen herbal mixture on volunteers post intra nasal route of administration.

H_a: Significant differences exist between the four indicators which are, the general overall Joseleen herbal mixture rating per in/outpatients who volunteered, prophylactic experience of volunteers, curative experience, and side effects of Joseleen herbal mixture on volunteers post intra nasal route of administration

RESULT

Table-1

Patient	Joseleen Herbal Mixture Rating	Positive or Negative Change in Symptom	Interval of Change	Favourable or Unfavorable Comment	Side Effects Noticed	Sex	Purpose
A	Very Good	Positive	Five Days	Favourable	None	Male	Curative
B	Good	Positive	Four Days	Favourable	None	Male	Curative
C	Excellent	Positive	Thirty Days Living With Covid 19 Infected Patients	Favourable	None	Male	Prophylaxis
D	Very Good	Positive	Five Days	Favourable	Extremely Slight Headache	Male	Curative
E	Very Good	Positive	Five Days	Favourable	None	Female	Curative
F	Very Good	Positive	Seven Days	Favourable	None	Male	Curative
G	Very Good	Positive	Twenty-Five Days Living With COVID 19 Infected Patients	Favourable	None	Female	Prohylaxis
H	Very Good	Positive	Five Days	Favourable	None	Female	Curative
I	Very Good	Positive	Four Days	Favourable	None	Male	Curative
J	Good	Positive	Sixty Days Living With COVID 19 Infected Patients	Favourable	None	Male	Prophylaxis
K	Not Ratable as There was No Result Seen From NCDC Laboratory	Indifferece	None	Neutral Stand	No Result	Male	Prophylaxis
L	Good	Positive	Seven Days	Favourable	Slight Headache	Male	Curative

Table-2: Indicator I; Overall Joseleen Herbal Mixture

Ratings	Marks	% Rating	A	B	C	D	E	F	G	H	I	J	K	L	Average Rating (%)
Excellent	30 (Maximum)	100	-	-	30	-	-	-	-	-	-	-	-	-	100
Very Good	25	83.3	25	-	-	25	25	25	25	25	25			-	83.3
Good	20	66.7	-	20	-	-	-	-	-	-	-	20	-	20	66.0
Average	15	50.0	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Poor	10	33.3	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Very Poor	5	16.7	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Not Ratable	0	-	-	-	-	-	-	-	-	-	-	-	0	-	0

Overall Average Rating of Joseleen Herbal Mixture = 83.1 %



Fig-1: Joseleen Herbal lipids Mixture ratings

Table-3: Indicator 2, Prophylactic Experience of Patients

Length Of Days with Covid Patients	Marks Rating	A	B	C	D	E	F	G	H	I	J	K	L	Average Rating (%)
≤ 10	0	NA	NA	-	NA	NA	NA	-	NA	NA	-	-	NA	NA
≥ 10 - ≤ 20	20	NA	NA	-	NA	NA	NA	25	NA	NA	-	-	NA	83.3
≥ 20 - ≤ 30	30	NA	NA	30	NA	NA	NA	-	NA	NA	-	-	-	100
≥ 30 - ≤ 40	30	NA	NA	-	NA	NA	NA	-	NA	NA	-	-	NA	NA
≥ 40 - ≤ 50	30	NA	NA	-	NA	NA	NA	-	NA	NA	-	-	NA	NA
≥ 50 - ≤ 60	30	NA	NA	-	NA	NA	NA	-	NA	NA	30	-	NA	100
≥ 60 DAYS WITH COVID 19 PATIENS	-	-	-	-	-	-	-	-	-	-	-	-	-	0

Overall Average Prophylactic Percentage Performance = 94.43 %

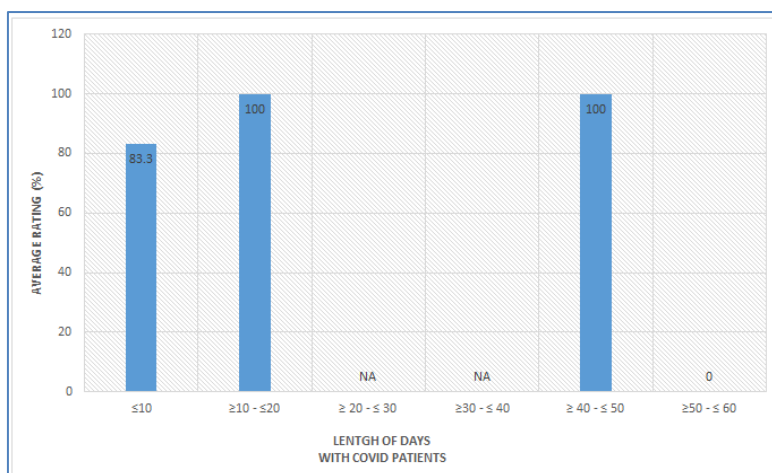


Fig-2: Average ratings in% versus length of days of interaction with covid19 patients

Table-4: Indicator 3, Curative Experience

Lentgh of Days of Drastic Change in Symptoms / Curative Experience (Negative Test)	Marks Rating	A	B	C	D	E	F	G	H	I	J	K	L	Average Rating (%)
≤ 2	30	-	-	NA	-	-	-	NA	-	-	NA	NA	-	NA / -
≥ 2 - ≤ 4	30	-	30	NA	-	-	-	NA	-	-	NA	NA	-	100
≥ 4 - ≤ 6	30	30	-	NA	30	30	-	NA	30	30	NA	NA	-	100
≥6 - ≤ 8	30	-	-	NA	-	-	30	NA	-	-	NA	NA	-	100
≥ 8 - ≤ 10	25	-	-	NA	-	-	-	NA	-	-	NA	NA	-	NA / -
≥10 - ≤ 12	20	-	-	NA	-	-	-	NA	-	-	NA	NA	-	NA/-
≥ 12 Days and Over	15	-	-	NA	-	-	-	-	-	-	-	-	-	NA /-
Overall Average Percentage Curative Experience for The Twelve Patients Tested Within one Week = 100%														

KEY

- ≥ 4DAYS - ≤ 7 DAYS = 30 MARKS (MAXIMUM MARKS OBTAINABLE)
- ≥ 7 DAYS - ≤ 10 DAYS = 25 MARKS
- ≥ 10 DAYS - ≤ 13 DAYS = 15 MARKS



Fig-3: Length of days of drastic change in symptoms versus average curative ratings in %

Table-5: Indicator 4 Side Effects Of Joseleen Herbal Mixture

Comment	Ratings	Marks Rating	A	B	C	D	E	F	G	H	I	J	K	L	Average Rating (%)
None	Excellent	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Very Mild	Very Good	05	-	-	-	5	-	-	-	-	-	-	-	-	17
Mild	Good	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Very Slight	Very Good	05	-	-	-	-	-	-	-	-	-	-	-	5	17
Slight	Good	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Very Severe	Very Poor	10	-	-	-	-	-	-	-	-	-	-	-	-	-
Severe	Poor	15	-	-	-	-	-	-	-	-	-	-	-	-	-
Overall Mean Side Effect Rating of Joseleen Herbal Mixture = 11%															

Table-6: Analysis of Result Using the Anova Technique

Indicator I %	Indicator II %	Indicator III %	Indicator IV %
100	83.3	100	0
83.3	100	100	17
66	100	100	17
Average	94.43	100	11

INDICATOR I

General Ratings of Joseleen Herbal Mixture %

INDICATOR II

Prophylactic Experience of Volunteers after 20.60 Days of Interaction with COVID 19 Patients

INDICATOR III

Curative Experience of Those Volunteers Infected With COVID 19 Disease Post Joseleen Herbal Mixture Administrations.

INDICATOR IV

Side Effects of Post Administration of Joseleen Herbal Mixture on Volunteers

ANOVA**Total Sum of Squares = 18,371****Indicator Sum of Squares = 17,413.65****Error Sum of Squares = Total Sum of Squares –****Indicator Sum of Squares = 957.45****Table-7: Anova Table**

Squares Of Variance (DF)		Sum of Squares	Mean Sum of Squares	F-Ratio
Between The Indicators	4-1 = 3	17,413.65	5,804.5	5804.5/119.68
Error	11-3 = 8	957.45	119.68	= 48.5
Total	12-1 = 11			

Tabulated Value = 4.07

Calculated Value = 48.5

Since calculated value far exceeded the value tabulated, significant differences thus exist between the four indicators at 95% confidence limits. We have no

enough evidence to reject the alternative hypothesis, hence it is retained.

Case fatality rate = $\frac{\text{Number of deaths due to COVID 19 among volunteers}}{\text{Number of cases of the same COVID 19 pandemic}} \times 100$

Case fatality rate, CFR = $0/40 \times 100 = 0\%$

DISCUSSION

We initially proposed Joseleen Herbal Mixture as a prophylactic agent for COVID19 disease, following its high potential for protecting individuals against Coronavirus infection, as evidenced from haemagglutination inhibition assay carried out using influenza virus, -- a closely related virus with 80% sequence homology [16, 17]. All the four members in the four families tested for prophylaxis remained negative, although one communicated us just through the telephone. However, the public health (Epidemiological Number codes), for the other three were documented and used in this assay report for calculation. Randomized assay conducted on those eight patients infected with COVID 19 disease revealed its high therapeutic potential, after administering informed consent questionnaires to volunteers. The Four indicators used were analyzed using the Analysis of variance technique (ANOVA) in order to critically appraise the herbal mixture. These were, Percentage overall rating of the herbal Mixture by volunteers; Their

prophylactic experience with the herbal mixture after staying with COVID 19 patients for a period between 20 – 60 days; None of their immediate twenty four family members had any signs nor symptoms of Corona virus infection, confirming further its potency as a prophylactic agent against the virus infection. Curative experience of the eight volunteers who were infected initially with corona virus showed that they all turned negative between intervals of \geq four days to \leq seven days, which implied that the Joseleen herbal lipids mixture has awesome therapeutic potentials for corona virus infection. Side effects experience of these same group of volunteers showed that only one out of the volunteers who had the herbal lipids for prophylaxis and one for therapeutic purposes complained of very slight/mild headache which subsided few minutes later--further confirming its safety for both oral and intra nasal routes of administration. Table 5 showed that only very little side effects were evidenced from the usage of Joseleen herbal mixture. The outcomes showed, Percentage ratings of 83.1%, 94.43%, 100% and 17% respectively. Likewise, the alternative hypothesis which stated that significant differences actually existed

between the indicators was confirmed using the ANOVA (Analysis of Variance) statistics technique. There were significantly different percentage ratings among the first three indicators ($P \leq 0.05$). However, the side effect percentage rating was significantly lower than the other three indicators ($P \leq 0.05$).

CONCLUSION

The locally sourced lipid solvent has a significant neutralization titre against the influenza A virus strain "A" (A/PR/6/32) Virus" used in this assay. Having been a long term lipid well known for its potency all over Africa against Flu viruses and corona viruses it is suggested that it could be adapted as a prophylactic medication in the first line of defence against these diseases. As the virus is lysed due to its lipid coat in this locally sourced lipid solvent. Without attachment to a living host membrane, the virus cannot infect.

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