Preparation, characterization and antimicrobial potential of essential oil based nanoemulsion formulated with Saponin extract

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Abstract

Several biochemical constituent present in castor and olive oil makes it an efficient antimicrobial agent against human pathogens. The current investigation aims at assessing the antimicrobial potential of olive and castor oil nanoemulsion formulated using aqueous saponin extract. Extraction protocol for saponin from Sapindus mukorossi was standardized. Saponin was characterized through FTIR. Aqueous extraction of S.mukorossi (0.4%) was used as biosurfactant for nanoemulsification of Olive and Castor oil through ultrasonication technique. The formulated nanoemulsion were investigated for their antimicrobial activity against four human pathogens namely, S.aureus, A.buamanni, B.subtilis, E.faecalis. Inhibitory concentration of the prepared nanoemulsions were determined and minimum inhibitory concentration value for Castor Oil nanoemulsion was found to be 16µl/ml and that for Olive Oil nanoemulsion was 128µl/ml. Sapindus mukorossi can thus emerge as potential source of biosurfactant for formulating emulsion based preparations in food industry.

Index Terms – Nanoemulsion , Saponin extract, Antimicrobial activity , Minimum inhibitory concentration ,

1. INTRODUCTION

Plant Origin essential oils are widely used against human pathogens causing various diseases. Essential oil are volatile molecules which are obtained from different part of plants as secondary metabolites. Essential oils extracted from edible plants are generally not harmful and hence can be potential source of food additives. There is an increasing demand for accurate knowledge of the minimum inhibitory (effective) concentrations (MIC) of essential oils to enable a balance between the sensory acceptability and antimicrobial efficacy. This can be achieved with in vitro and in vivo studies. The use of edible nanoemulsion as a method to disperse lipophilic active ingredients in an aqueous media, is emerging as potential tool as antimicrobial agent. Nanoemulsions are colloidal dispersions, composed of an oil phase, aqueous phase, surfactant at appropriate ratio. Nanoemulsion of essential oils is reported to further improve their efficacy due to their distinct physio-chemical and functional characteristics. Nanoemulsions are also used to enhanced transport of bioactive constituents through biological membrane, thus intensifying the bioavailability and antimicrobial activity against pathogens. Nanoemulsion of bioactive compounds also offers larger resistance to destabilization phenomena such as particle aggregation or gravitational separation. The present study describes i)saponin extraction protocol from S.mukorossi fruit pericarp powder ii)nanoemulsification of castor and javas oil using extracted saponin iii)measurement of physical properties of oil and oil added with synthetic and bio surfactant iv)antimicrobial evaluation of developed nanoemulsion against four human pathogens namely, S.aureus, A.buamanni, B.subtilis, E.faecalis. All the mentioned pathogens are source of various disease in humans.

2. Theoretical Background

2.1 Essential Oil : An essential oil is a concentrated hydrophobic liquid containing volatile (easily evaporated at normal temperatures) chemical compounds from plants. Essential oils are also known as volatile oils, ethereal oils, aetherolea, or simply as the oil of the plant from which they were extracted, such as oil of clove. An essential oil is "essential" in the sense that it contains the "essence of" the plant's fragrance; the characteristic fragrance of the plant from which it is derived. Essential oils are generally extracted by distillation, often by using steam. Other processes include solvent extraction, absolute oil extraction, resin tapping, wax embedding and cold pressing. Essential oils are used in perfumes, cosmetics, soaps ,flavouring food and drink, adding scents to incense.

Essential Oils used:

Olive oil: Olive oil is a fat obtained from the fruit of the Olea europaea (olive tree), a traditional tree crop of the Mediterranean region.

Castor oil: Castor oil is a multi-purpose vegetable oil that people have used for thousands of years.It's made by extracting oil from the seeds of the Ricinus communis plant.

2.2Surfactant: Surfactants are compounds that lower the surface tension (or interfacial tension) between two liquids, between a gas and a liquid, or between a liquid and a solid. Surfactants may act as detergents, wetting agents, emulsifiers, foaming agents, and dispersants. Surfactants are organic compounds that are amphiphilic in nature, that means they contain both hydrophobic groups (their tails) and hydrophilic groups (their heads).Therefore, a surfactant contains both a water-insoluble (or oil-soluble) component and a water-soluble component. Surfactants will diffuse in water and adsorb at interfaces between air and water or at the interface between oil and water, in the case where water is mixed with oil. The water insoluble hydrophobic group may extend out of the bulk water phase, into the air or into the oil phase, while the water-soluble head group remains in the water phase.

Types of surfactant: Anionic surfactants, Cationic Surfactants, Non-ionic surfactant, Amphoteric Surfactant, Biosurfactant

Surfactant used in Nanoemulsion:

Saponin extracted from Sapindus Mukrossi Fruit Pericarp Powder.

Saponin: saponins are a class of chemical compounds found in particular abundance in various plant species. More specifically, they are amphipathicglycosides grouped phenomenologically by the soap-like foam they produce when shaken in aqueous solutions, and structurally by having one or more hydrophilic glycoside moieties combined with a lipophilic triterpene or steroid derivative. The amphipathic nature of saponins gives them activity as surfactants with potential ability to interact with cell membrane components, such as cholesterol and phospholipids, possibly making saponins useful for development of cosmetics and drugs.

Standard Surfactant Used:

1.Sodium Dodecyl Sulphate: Sodium dodecyl sulfate (SDS) is an anionic (negatively charged) detergent. SDS is in the family of organosulfate compounds, and has the formula, CH 2 11 SO 4 Na. It consists of a 12-carbon tail attached to a sulfate group, that is, it is the sodium salt of a 12-carbon alcohol that has been esterified to sulfuric acid. An alternative description is that it is an alkyl group with a pendant, terminal sulfate group attached. As a result of its hydrocarbon tail, and its anionic "head group", it has amphiphilic properties that allow it to form micelles, and so act as a detergent. The critical micelle concentration (CMC) in pure water at 25 °C is 8.2 mM, and the aggregation number at this concentration is usually considered to be about 62.

2.Cetyltrimethylammonium bromide(CTAB): is a quaternary ammonium surfactant. It is one of components of the topical antiseptic cetrimide. The cetrimonium (hexadecyltrimethylammonium) cation is an effective antiseptic agent against bacteria and fungi. CTAB, due to its relatively high cost, is typically only used in select cosmetics. CTAB forms micelles in aqueous solutions. At 303 K (30 °C) it forms micelles with aggregation number 75-120

2.3.Nanoemulsion: Nanoemulsions are colloidal dispersions which are composed of an oil phase, aqueous phase, surfactant and co surfactant at appropriate ratios. Unlike coarse emulsions micronized with external energy. Nanoemulsions are based on low interfacial tension. This is achieved by adding a cosurfactant, which leads to spontaneous formation of a thermodynamically stable nanoemulsion. The term 'Nanoemulsions' is often used to designate emulsions with the internal phase droplets smaller than 1000 nm. The Nanoemulsions are also referred as mini emulsions, ultrafine emulsions and submicron emulsions. They can be prepared simply by blending oil, water, surfactant, and cosurfactant, in the right proportion, with mild agitation.

Main three components of Nanoemulsions are as follows: • Oil • Surfactant • Aqueous phasare different types of microbes: bacteria fungi algae protozoa viruses.

2.4Pathogen: Pathogens are anything that can produce disease. A pathogen may also be referred to as an infectious agent, or simply a germ.

Pathogens used:

1. Staphylococcus aureus: It is a Gram-positive, round-shaped bacterium that is a member of the Firmicutes, and it is a usual member of the microbiota of the body, frequently found in the upper respiratory tract and on the skin.

2. Acinetobacter baumannii: It is a typically short, almost round, rod-shaped (coccobacillus) Gram-negative bacterium.

3. Bacillus subtilis:It is known also as the hay bacillus or grass bacillus, is a Gram positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans.

4. Enterococcus faecalis: It was formerly classified as part of the group D Streptococcus system – is a Gram-positive, commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals.

2.5Antimicrobial Activity: An antimicrobial is an agent that kills microorganisms or stops their growth. Antimicrobial medicines can be grouped according to the microorganisms they act primarily against. Agents that kill microbes are called microbicidal, while agents that merely inhibit their growth are called biostatic. The main classes of antimicrobial agents are disinfectants; which kill a wide range of microbes on non-living surfaces to prevent the spread of illness, antiseptics which can be applied to living tissue and help reduce infection during surgery and antibiotics ;which destroy microorganisms within the body.

Minimum Inhibitory concentration (MIC): Minimum inhibitory concentrations (MICs) are defined as the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism after overnight incubation, and minimum bactericidal concentrations (MBCs) as the lowest concentration of antimicrobial that will prevent the growth of an organism after subculture on to antibiotic-free media. MICs are used by diagnostic laboratories mainly to confirm resistance, but most often as a research tool to determine the in vitro activity of new antimicrobials, and data from such studies have been used to determine MIC breakpoints.

Methods to determine MIC

1)Agar dilution: This method used by researchers to determine the Minimum Inhibitory Concentration (MIC) of antibiotics. It is the dilution method most frequently used to test the effectiveness of new antibiotics when a few antibiotics are tested against a large panel of different bacteria.

2)Broth microdilution: Broth microdilution is a method used for testing the susceptibility of bacteria to antibiotics.

2.5Physical Properties :

1.Contact angle: When an interface exists in between a liquid and solid, the angle between the surface of the liquid and the outline of the contact surface is described as the contact angle θ . The contact angle is a measure of the wettability of solid by a liquid. In the case of complete spreading, the contact angle is 0°. Between 0° and 90°, the solid is wettable and above 90° it is not wettable. In the case of ultra hydrophobic materials with the so-called lotus effect, the contact angle approaches the theoretical limit of 180°.

2.Contact angle: When an interface exists between a liquid and a solid, the angle between the surface of the liquid and the outline of the contact surface is described as the contact angle θ (lower case theta). The contact angle (wetting angle) is a measure of the wettability of a solid by a liquid. In the case of complete wetting (spreading), the contact angle is 0°. Between 0° and 90°, the solid is wettable and above 90° it is not wettable. In the case of ultra hydrophobic materials with the so-called lotus effect, the contact angle approaches the theoretical limit of 180°.

3.Interfacial Tension: Interfacial tension is defined as the work which must be expended to increase the size of the interface between two adjacent phases which do not mix completely with one another. In other sense the term relates to the liquid/liquid and liquid/solid phase boundaries, while for the liquid/gaseous interface it is refered as surface tension and for the solid/gaseous interface it is refered as surface free energy. As a measure of work per unit area or force per wetted length, interfacial tension has the units mN/m and is designated by the symbol σ or γ.

Material Used:

For Saponin extraction: Sapindus mukorossi fruit pericarp powder

1. Deionized water 2.Magnetic stirrer 3.Beaker 4.Magnetic needle 5.Lyophilizer machine 6.Refrigerator 7. Whatman filter paper number 1

For nanoemulsion:

1. Essential oils (Olive, Castor) 2. Distilled water 3. Extracted saponin 4. Ultrasonicator 5.Magnetic stirrer

6. Magnetic needle
For physical properties measurements: 1.Goniometer (for contact angle , surface tension, interfacial tension measurements) 2.Weighing machine
For biological studies: 1**)** Laminar air flow (LAF) 2.Autoclave 3.Microtiter plate 4.Nutrient Broth medium 5.Sugar tubes 6.Cotton plugs

Human pathogens used:

1.Staphylococcus aureus 2.Acinetobacter baumannii 3.Bacillus subtilis 4.Enterococcus faecalis

EXPERIMENTAL WORK :

1. **Extraction of saponin from sapindus mukorossi fruit pericarp powder**

To find effect of extraction technique on the quality of biosurfuctant, the dried powder of sapindus mukorossi was extracted using single solvent water that is deionized water. 20gm of S.mukorossi fruit pericarp powder was mixed with 100 ml deionized water. For homogenous mixing the solution was stirred using magnetic stirrer for 120 minutes. Then the macro particles of solution were allowed to settle down for 10-15 minutes. Supernatant was filtered using vacuum filtration with whatman filter paper number 1 .This procedure wasrepeated twice using the residual to obtain maximum extraction of saponin from the powder. The obtained clear filtrate was frozen for 24 hr, then subjected to freeze drying by using lyophilizer. Powder has obtained was ground in pestle and mortar to obtain free flowing powderfor further use and characterization.

2. Preparation of Nanoemulsion

Nanoemulsions of essential oil is prepared by employing ultrasonication technique using aqueous saponin extaract. Primary emulsions were prepared by mixing essential oil(0.5% v/v) and saponin extract (0.4% w/w) in deionized water and the mixture was stirred using magnetic stirrer for 20 minutes. These primary emulsions were then nanoemulsified through ultrasonicator for 20 minutes. The obtained nanoemulsions were used for characterization and bioefficacy testing.

Physical property of Essentail oils:

Surface Tension:

Oil	**Surface Tension**
Castor	33.83 mN/m
Olive	34.24 mN/m

Interfacial Tension:

Oil	**Interfacial Tension**
Castor	14 mN/m
Olive	5.32 mN/m

Contact Angle:

Oil	**Contact Angle (in degrees)**
Castor on glass	46.75
Castor on parafilm	47.6
Castor on teflon	56.95
Castor on pdms	65.75
Oil	Contact Angle (in degrees)
Olive on glass	40.4
Olive on parafilm	44.55
Olive on teflon	57.75
Olive on pdms	63.9

1. **Contact angle by adding SDS.**

Critical micelle concentration of sds:8mM

SDS to be added in oil was calculated using formula:

(molarity of solution*molecular weight of SDS*ml of solution)/**1000**Molecular weight of SDS=288.37 gm/mole
=(0.008*288.37*10)/1000

=0.0230 gm of sds was added in **10 ml** of oil

Oil	**Contact angle(in degrees)**
Castor on glass	14
Castor on parafilm	27.9
Castor on Teflon	31.35
Castor on pdms	31.1

2) Contact angle by adding CTAB

CTAb to be mixed with oil was also calculated with the same formula.Critical micelle concentration of ctab=1mM
CTAB in gm= 0.001*364.45*10/1000

=0.00364 gm of ctab was added in 10 ml of oils.

Oil	**Contact angle(in degrees)**
Castor on glass	10.5
Castor on parafilm	17.3
Castor on Teflon	39.9
Castor on pdms	40.05
Oil	Contact angle(in degrees)
Olive on glass	7.4
Olive on parafilm	11.5
Olive on Teflon	7.3
Olive on pdms	10.5

3)Contact angle were also measured by adding saponin.

Saponin to be mixed with oil was also calculated with the same formula.Critical micelle concentration of saponin=0.017mM
Saponin in gm= 0.017*634.851*10/1000

=0.1079 gm of ctab was added in 10 ml of oils

Oil	**Contact angle (in degrees)**
Castor on glass	13
Castor on parafilm	64.9
Castor on Teflon	89.55
Castor on pdms	58.85

Oil	**Contact angle (in degrees)**
Olive on glass	12.1
Olive on parafilm	50.25
Olive on Teflon	58.1
Olive on pdms	33.2

Antibacterial assay

The human pathogens were subjected to growth in nutrient broth for 24 hours.To evaluate the antimicrobial activities of nanoemulsion, The MIC values of essential oils were evaluated using broth microdilution assay in sterile 96-well microtiter plates in duplicates. The procedure involves involves preparing serial dilutions of antimicrobial agent(nanoemulsion) in distilled water (1024,512,256,128,64,32,16,8,4 µl/ml) in a 150 µl mueller hinton broth dispensed in 96-well microtitration plate containing. Then each well is inoculated with a microbial inoculum prepared in nutrient broth after dilution of standardized microbial suspension adjusted to 0.1 Mcfarland scale(10^8 cells).After well mixing the 96 well microtitration plate were incubated under suitable conditions

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Microtitration plate

Pathogens **Cultured pathogens**

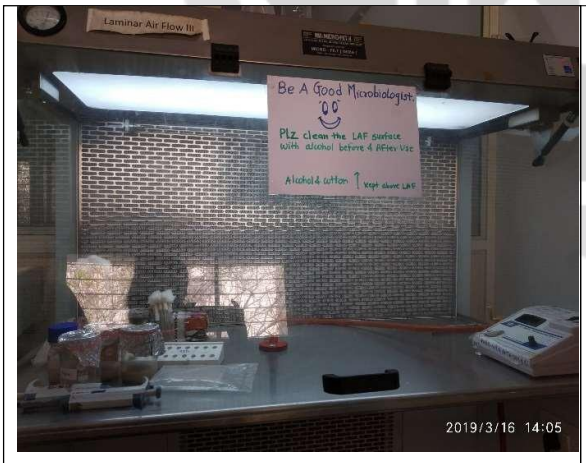


Figure 39:Laminar Air Flow

Photocalorimeter

3. Results

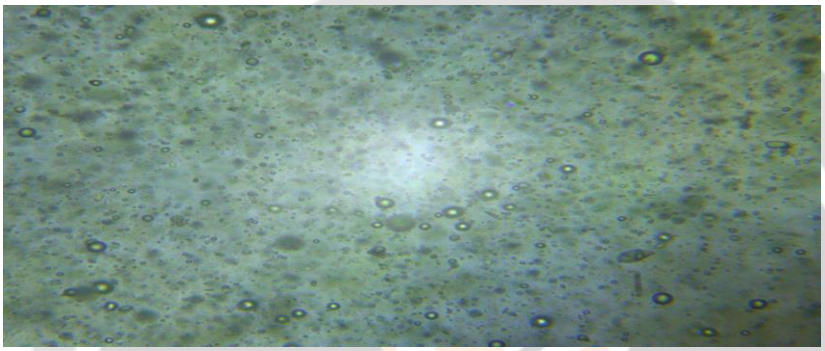
Fourier Transform Infrared Spectroscopy

Aqueous extract of saponin gave major band absorption at 3422 cm^{-1} (O-H stretch of -OH group present in glycon part),2927 cm^{-1} (C-H stretch of alkyl group present in glycon and aglycon part), 1735 cm^{-1} (C=O stretch of carbonyl group present in aglycon part) 1654cm^{-1} (C=C stretch of alkanyl group present in the aglycon part), 1458cm-1 (C-H bending of methylene group present in the aglycon part),1376cm^{-1} (C-H bending of methyl group presentin the glycon part), 1259cm^{-1}(C-O stretch of ester linkage present in the aglycon part and glyconpart.

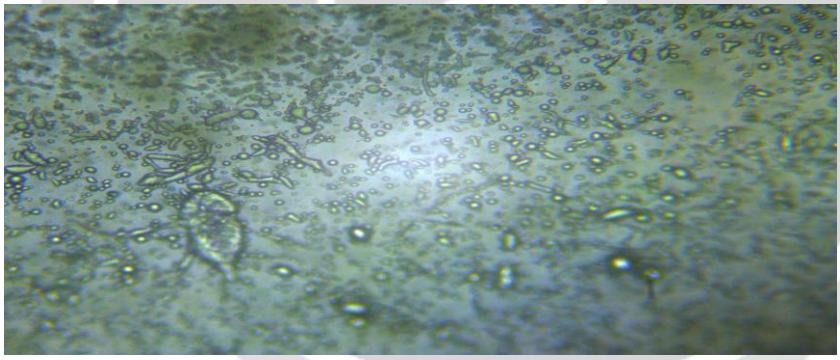
Microscopic images

Images of Nanoemulsion were taken using Compound Microscope

Castor Nanoemulsion



Olive nanoemulsion



The MIC values of essential oil nanoemulsions were evaluated using broth microdilution assay in sterile

Determination of Minimum Inhibitory Concentration(MIC):

96-well microtiter plates in duplicates.

Positive control:Muller Hinton Broth(150µl)+Pathogens(2µl)

Results for inhibitory cuncentrations are obtained in terms of Optical Density:

Optical Density:

The ability of a laboratory specimen to absorb or block the passage of light. The optical density of a laboratory sample can beused as an indicator of the concentration of specific compone nts in the sample.

The obtained Optical density were further used for calculating minimum inhibitory concentration using

the following formula.

Formula:% Survival=(OD at given concentration-OD at Negative Control)/OD at positive control

% Inhibition=100-(%Survival)

Results for Castor Oil Nanoemulsion For first plate.

1.Optical Density values

Bacteria	*S.aureus*	*A.baumanni*	*B.subtilis*	*E.faecalis*	
Positive Control	0.5839	0.2876	0.2557	0.7268	Negative Control Castor
Concentration(in µl/ml)					
1024	1.0379	0.8423	0.7782	0.7155	0.803
512	0.7882	0.7162	0.5918	0.9417	0.5445
256	0.7308	0.8657	0.7485	0.8387	0.8065
128	0.5967	0.549	0.278	0.7074	0.8488
64	0.2047	0.1542	0.7975	0.9812	0.1969
32	0.4903	0.5143	0.2399	0.9424	0.128
16	0.528	0.2192	0.5377	0.9436	0.7792
8	0.4792	0.2327	0.2603	0.9852	0.0897
4	0.5118	0.2318	0.2343	0.9435	0.1537

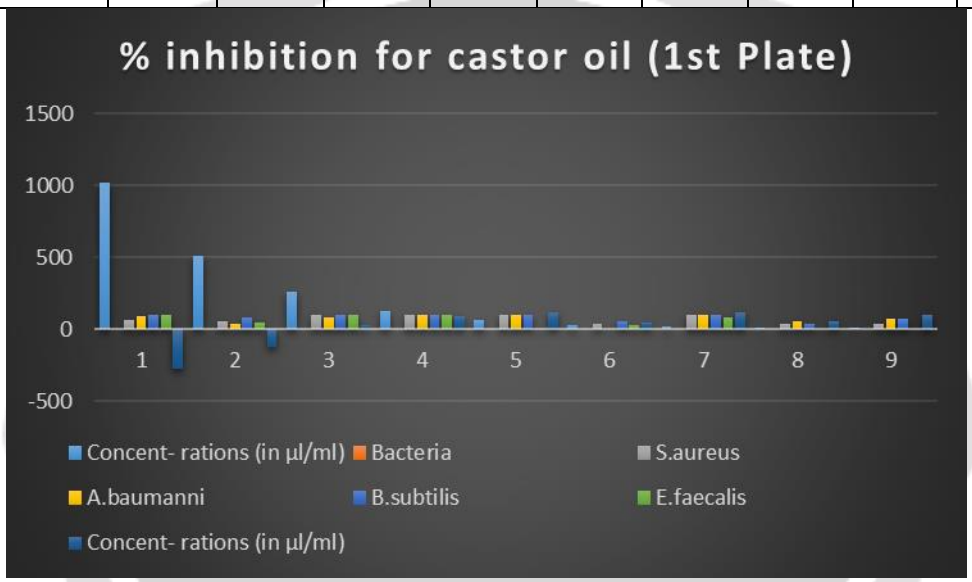
Inhibitory concentration values for Castor oil Nanoemulsion

% Survival For castor nanoemulsion

Concent-rations (in µl/ml)	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	40.22	41.73	0	0	1.33	62.04	0	66.70	61.32
A.baumanni	13.66	59.70	20.58	0	0	134.31	0	49.72	27.15
B.subtilis	0	18.49	0	0	234.8	43.76	0	66.71	31.52
E.faecalis	0	54.65	4.43	0	107.91	74.36	22.61	132.2	108.6

% Inhibition for castor nanoemulsion

Concent-rations (in µl/ml)	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	59.78	58.27	100	100	98.76	37.96	**100**	33.3	38.68
A.baumanni	86.34	40.3	79.42	100	100	-	**100**	50.28	72.85
B.subtilis	100	81.51	100	100	100	56.24	**100**	33.29	68.48
E.faecalis	100	45.35	95.57	100	-	25.64	77.39	-	-



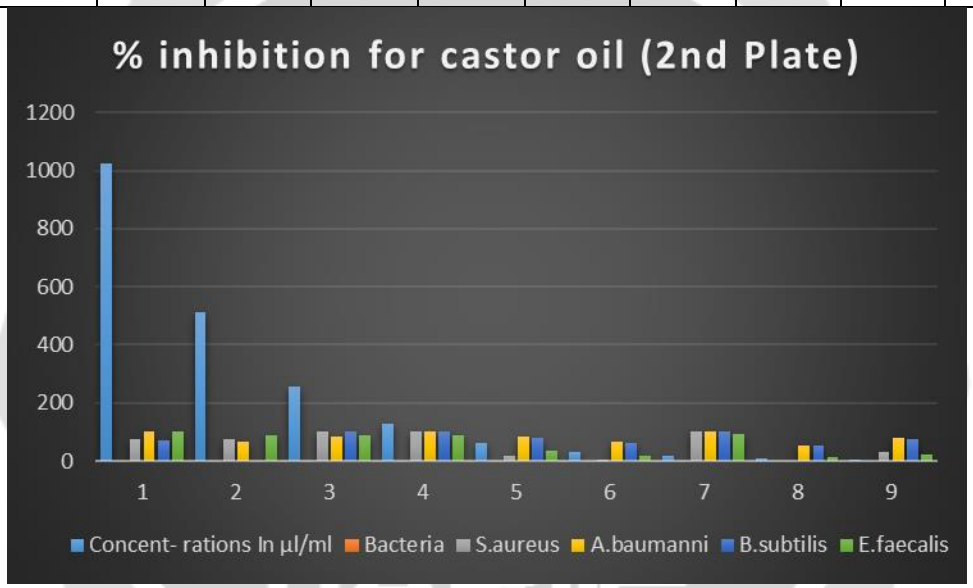
1. Result for Castor Oil Nanoemulsion for Second plate
Optical Density values

	Bacteria				
	S.aureus	*A.baumanni*	*B.subtilis*	*E.faecalis*	
Positive Control	0.5434	0.2398	0.3208	0.9918	Negative control
Concentration (in µl/ml)					
1024	0.9418	0.6908	0.8963	0.3949	0.803
512	0.684	0.6225	0.9777	0.6705	0.5445
256	0.677	0.8402	0.593	0.9361	0.8065
128	0.596	0.2854	0.2814	0.955	0.8488
64	0.6391	0.2393	0.2665	0.8444	0.1969

32	0.6566	0.2131	0.2557	0.9282	0.128
16	0.636	0.2003	0.2332	0.8684	0.7792
8	0.7132	0.2022	0.245	0.0.9377	0.0897
4	0.5204	0.2042	0.2377	0.9021	0.1537

% Inhibition

Concent-rations In µl/ml	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	74.46	74.33	100	100	18.63	2.73	**100**	-	32.52
A.baumanni	100	67.48	85.95	100	82.32	64.52	**100**	53.09	78.95
B.subtilis	70.92	-	100	100	78.31	60.2	**100**	51.59	73.82
E.faecalis	100	87.28	86.94	89.3	34.72	19.32	91.01	14.5	20.96



In the both plates nanoemulsion has inhibited growth of pathogens at various concentration andminimum concentration at which nanoemulsion inhibited growth of pathogens is **16 µl/ml.**

Thus MIC value for Castor Oil Nanoemulsion is **16 µl/ml.**

1. **Result for Olive oil Nanoemulsion for first plate:**

Optical density values

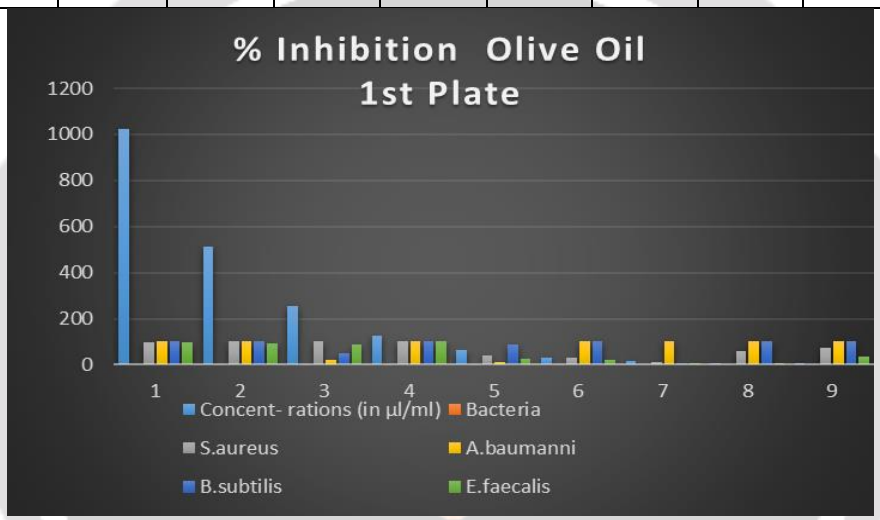
Bacteria	*S.aureus*	*A.baumanni*	*B.subtilis*	*E.faecalis*	
Positive Control					Negative Control olive
Concentration (in µl/ml)	0.4807	0.502	0.3155	0.7351	
1024	0.9736	0.7771	0.8425	0.9758	0.9615
512	0.7986	0.8469	0.6344	0.9346	0.8657
256	0.6946	1.122	0.8802	0.8314	0.7275
128	0.5116	0.6033	0.3957	0.6779	0.856
64	0.6142	0.7584	0.3489	0.8501	0.3162
32	0.6685	0.266	0.264	0.9175	0.3363
16	0.6947	0.2168	0.5693	0.9694	0.2588
8	0.5033	0.2034	0.2564	0.9679	0.2977
4	0.4679	0.213	0.257	0.7945	0.3322

%Survival for olive oil nanoemulsion

Concent-rations In µl/ml	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	2.51	0	0	0	61.99	69.10	90.68	42.77	28.22
A.baumanni	0	0	78.58	0	88.08	0	0	0	0
B.subtilis	0	0	48.39	0	10.36	0	98.4`	0	0
E.faecalis	1.94	9.37	14.13	0	72.62	79.06	96.66	91.17	62.88

%Inhibition

Concent-rations (in µl/ml)	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	97.49	100	100	**100**	38.01	30.9	9.32	57.23	71.78
A.baumanni	100	100	21.42	**100**	11.92	100	100	100	100
B.subtilis	100	100	51.61	**100**	89.64	100	1.59	100	100
E.faecalis	98.06	90.63	85.87	**100**	27.38	20.94	3.34	8.83	37.12



2. Result for olive oil Nanoemulsion for second plate

Bacteria	*S.aureus*	*A.baumanni*	*B.subtilis*	*E.faecalis*	
Positive Control	0.4729	0.2436	0.3675	0.7705	Negative control
Concentration(in µl/ml)					
1024	0.8657	1.0516	0.8792	0.9877	0.9615
512	0.7746	0.5826	0.7406	1.0004	0.8657
256	0.5816	0.7559	0.747	0.6974	0.7275
128	0.5986	0.8052	0.5197	0.6322	0.856
64	0.6114	0.2397	0.2946	0.8831	0.3162
32	0.7998	0.2371	0.6754	0.8831	0.3363
16	0.5168	0.313	0.4736	0.9401	0.2588

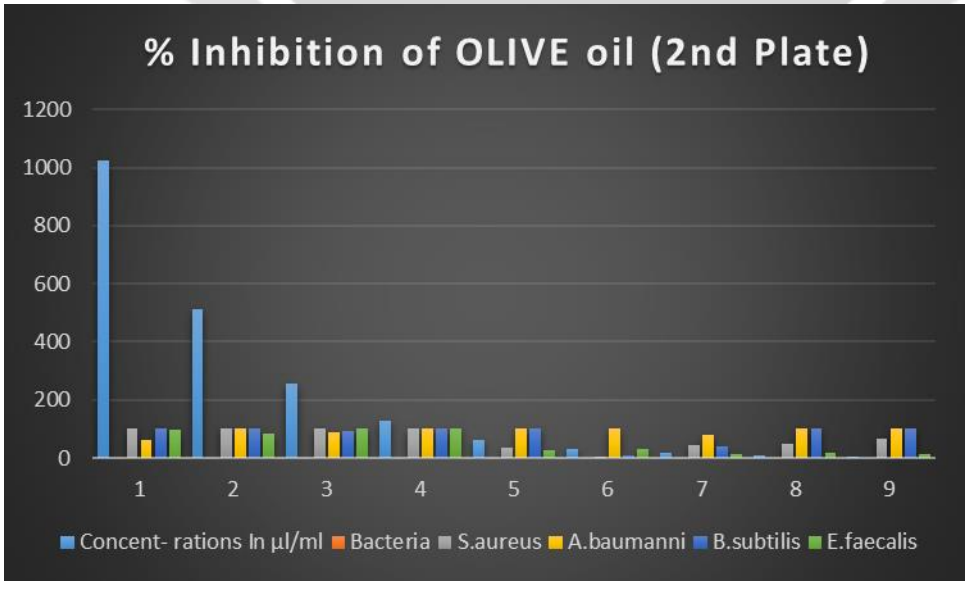
0.	0.5506	0.2027	0.2518	0.9149	0.2977
4	0.4946	0.187	0.2583	0.9839	0.3322

% Survival for olive oil nanoemulsion

Concent-rations (in µl/ml)	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	0	0	0	0	63.05	98.01	54.55	53.47	34.34
A.baumanni	36.98	0	11.65	0	0	0	22.24	0	0
B.subtilis	0	0	5.30	0	0	92.27	58.44	0	0
E.faecalis	3.40	17.48	0	0	73.57	70.96	88.42	80.10	84.54

% Inhibition

Concent -rations In µl/ml	1024	512	256	128	64	32	16	8	4
Bacteria									
S.aureus	100	100	100	**100**	36.95	1.99	45.45	46.53	65.66
A.baumanni	63.02	100	88.35	**100**	100	100	77.76	100	100
B.subtilis	100	100	94.7	**100**	100	7.73	41.56	100	100
E.faecalis	96.6	82.52	100	**100**	26.43	29.04	11.58	19.9	15.46



In the both plates nanoemulsion has inhibited growth of pathogens at various concentrationand minimum concentration at which nanoemulsion inhibited growth of pathogens is **128 µl/ml.**

Thus MIC value for olive Oil Nanoemulsion is **128 µl/ml.**

FINAL RESULT:

Sr. No.	Oil	MIC Value (µl/ml)
1	**Castor oil**	**16 µl/ml**
2	**Olive oil**	**128 µl/ml**

4. CONCLUSIONS

1) Saponin extracted from the fruit pericarp powder of S.mukorossi using 100 % aqueous solvent is used for nanoemlsification of Castor and Olive Oil. Primary nanoemulsions were prepared using magnetic stirrer and ultrasonication technique was used to prepare stable nanoemulsions of castor and olive oil.

2) The developed nanoemulsions were tested for antimicrobial activity against four human pathogens namely S.aureus, A.baumanni, B.subtilis, E.faecalis. The antimicrobial assay was carried out using Broth microdilution method. The developed nanoemulsions of Castor and Olive showed inhibitory activity at various concentrations.

3) The minimum inhibitory concentration was found to be 16 µl/ml and128 µl/ml for castor and olive oil nanoemulsion respectively.

4) Being eco-friendly and non -toxic, sapindus extract can serve as potential alternative to the synthetic surfactant for Nano emulsification purpose in various application in food industry.

6. REFERENCES

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