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Formulation of anti-microbial and anti-aging oil for topical application from natural resources

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Abstract
The naturally occurring resources like coconut oil, ginger extract, and vitamin E possess substantial beneficial effect on human skin. The phytochemicals of coconut oil and ginger extract have anti-oxidant and anti-microbial activities. In the present work, a value added virgin coconut oil was developed mixing ginger extract and vitamin E with it. The oil was tested for its efficacy against bacteria and fungi that are potent causative agents of skin infection in human beings. The oil described in this article, is rich in anti-oxidants and having anti-aging property. Regular use of this oil may protect the skin from any getting infected by microbes as well as prevent the skin from premature aging. The extraction of coconut oil, ginger and vitamin E from wheat germ was done by super critical fluid extraction in pilot scale, using carbon dioxide as solvent. The molecular docking was done to support the wet lab data of anti-microbial activities of this oil with in-silico analysis.

Keywords: virgin coconut oil, ginger extract, vitamin E, anti-microbial, anti-aging

1. Introduction
Coconut or *Cocos nucifera* (L.) (Arecaceae) is found abundantly in India and other parts of the world. Coconut is a popular fruit consumed by human in summers and serves as an important source of some important therapeutic agents. The chemical components of *C. nucifera* are used as anti-helminthic, anti-inflammatory, anti-nociceptive, antioxidant, antifungal, antimicrobial, anti-tumorogenic agent (Lima *et al*., 2015). Coconut is used in India as fruit, source of edible as well as cosmetic oil, the leaves of coconut trees are used as roofing material, making toys, storage of rice, etc. In the present study, we prepare an anti-bacterial body oil that can prevent from skin diseases as well possesses natural anti-aging properties. Ginger extract and natural tocopherol was mixed in appropriate ratio with virgin coconut oil to develop this formulation. Plant extracts are known to be effective against skin diseases caused by microbial action and restore cutaneous homeostasis (Lin *et al*., 2017). The therapeutic benefits of plant extracts generally do not have side-effects like synthetic products and shows anti-inflammatory and antioxidant effects on the skin, promotes of wound healing, tissue repair and regeneration of skin barrier. Regular use of natural products made from medicinal plants protects the skin from allergens, irritants, infections and UV damage, thus prevents the skin from premature aging. Aloevera contains vitamin E which is a natural anti-oxidant and proven to have anti-aging properties. Like coconut and aloevera, ginger (*Zingiber officinale*) extracts are potent antioxidants and exhibits excellent anti-aging activity due to its ability to inhibit collagenase enzyme and thereby reducing the degradation of collagen. Strong anti-inflammatory properties of ginger makes it suitable for soothing dry, itchy, inflamed skin. It increases the circulation in the skin and aids in carrying essential nutrients, which helps to detoxify and promote overall healthier skin. In the present study, the physical parameters (colour, odour, stability, exposure to direct sunlight) of the oil developed by mixing plant extracts, were vividly studied. Anti-microbial property was studied in *Staphylococcus aureus* and *Epidermophyton floccosum* by cup disc method or Kirby–Bauer test. The preliminary data that are presented in this article is promising to develop an ant-microbial body oil with anti-aging properties through proper pre-clinical and clinical trials.

2. Materials and Methods
The virgin coconut oil was mixed with ginger extract in a specific ratio. There were two experimental sets, the first set was kept in laboratory condition and the second set was exposed to direct sunlight to study the stability of the oil. If the ingredients of the oil degrade in presence of sunlight, the stability will be disturbed which can be detected by UV-Vis spectrophotometer. Further, the stability data is supported by in-silico studies (Biovia_Discovery Studio). Each set was subdivided again into two sets, where, in one set vitamin E was added and in other set vitamin E was not added. The experiment was done in triplicates. Phytochemicals were extracted by supercritical fluid extraction pilot plant (Gram Tarang Foods Pvt. Ltd., Odisha, India).

2.1 Virgin coconut oil
There is a minute difference between raw and virgin coconut oil. Depending on the method of extraction, virgin coconut oil is exposed to heat, but raw coconut oil are never exposed to heat in any step of extraction process.
Coconut procured from the village Kanchili, Srikakulam District, Andhra Pradesh, India, was used in the present study and processed as per the method described by Zuknik *et al*., 2016. The grated coconut was sun-dried to lower the moisture to 2-3% as it is necessary to avoid clogging of the capillary restrictor. The dried sample was ground and sieved to get a particle size ranging from 0.5 to 1.0 mm. Carbon dioxide gas with purity of 99.95% was used as solvent in super critical fluid extraction process.

2.2 Ginger extract
The fresh ginger was procured from local market, sun-dried to lower the moisture content to 7-10% from 45-50%. It was ground and sieved to get particle size of 0.5 to 1.0 mm, same as described above in case of coconut.

2.3 Extraction of Natural Vitamin E from Wheat Germ
Extraction of vitamin E was done from wheat germ as described by Ge *et al*., 2002.Sample preparation includes heating raw wheat germ by far-infrared (IR) rays for eight minutes at 105°C. After 8 min, an additional heating period was necessary to reduce the water content of the material. The water content should be reduced from 4.30% to 11.50%. The dried material was ground and sieved to particle size of 0.13 to 2.1 mm.

2.4 Supercritical Fluid Extraction (SCFE) Process
Supercritical fluid extraction (SFE) utilizes supercritical fluid (carbon dioxide, in this case) as solvent. Carbon dioxide has similar solubility and diffusivity as of liquid and gas respectively, and several natural products are dissolved in it. Supercritical carbon dioxide is used to extract coconut oil, ginger extract and vitamin E because it has low critical temperature (31°C), selectivity, inertness, low cost, non-toxic, and capable to extract thermo labile compounds (Zhang *et al*., 2018). As the polarity of supercritical carbon dioxide is low, it is suitable for extraction of non-polar lipid and volatile oil. Sometimes, modifier may be supplemented tosuper critical carbon dioxide to increase its solvating properties significantly. SCFE was performed in Gram Tarang Foods Pvt. Ltd., Odisha. India.

2.5 Standardization of Oil Mixture
The ginger extract was highly concentrated and hence mixing the virgin coconut oil with ginger extract needed to be done in a specific ratio (v/v). The whole range of dilution factor, starting from 5x to 400x was checked and ultimately 400x dilution was standardised. The ginger extract was mixed with virgin coconut oil in the ratio of 1:400, i.e., 1 part of ginger extract was mixed with 399 parts of virgin coconut oil to get an uniform mixture with no visible precipitation. This mixture was infused with 1% vitamin E.

2.6 Determining the stability of mixture by UV-Vis Spectrophotometer
After the incubation period of 12 hrs, 24 hrs and 48 hrs in both sunlight and lab condition the value added coconut oil and the normal coconut oil were being analyzed in UV-Vis spectroscope to detect any abnormal changes in the oil due to the exposure of two different extreme conditions but found no significant changes.

2.7 Minimum Inhibitory Concentration (MIC)
Cup disc method or Kirby–Bauer test has found to be one of the most simple yet significant test for the measuring the zone of inhibition of the microbial growth due to any exposure (Brown and Kothari, 1975; Anon 1980). The number of the zone of inhibition has been confirmed from three parallel studies and taken as the mean value of the results with cup disc method with bacteria and fungi. These studies also were being compared with the known antimicrobial drugs available in the market for validation. The virgin coconut oil and the mixture of the coconut oil, ginger oil and vitamin E were taken in different concentration showed an average value of the zone of inhibition where the combination of these mixed compound has shown a maximum zone of inhibition. The lower concentration of the mixed drug has an effect on the bacterial and the fungal growth which has been measured by calculating the zone of inhibition and the values are (+/-) SD of three parallel measurements. The bacterial culture method has been shown in Figure 1 (a,b,c,d) are the different concentration of virgin coconut oil and the mixture of the coconut oil, ginger oil and vitamin E solutions.

2.8 In-silico Analysis
Various phytochemicals of Coconut oil and ginger oil have been listed and their respective SDF were taken accordingly from Pubchem, Molinstincts, and Chebi. The enzyme corresponding to microbe of Aster Yellow has been taken from BRENDA (Braunschweig Enzyme Database). Then, the PDB (Protein Data Bank) code was found from RCSB (Research Collaboratory for Structural Bioinformatics). The above mentioned information was then processed in Discovery Studio to initiate Docking. Table 1 and 2 are showing the list of the pharmacophores of coconut and the targeted genes and the PDB number of the bacteria and fungi respectively.

Table 1: The list of pharmacophores and the targeted genes from *S. aureus*

Sl. No	Phytochemicals of coconut	Targeted genes from *S. aureus*	PDB No of the Genes
1	lauric acid	toxin	4TW1
2	myristic acid	ligase	3IP4
3	linolenic acid	hydrolase inhibitor	3WDG

Table 2: The list of pharmacophores and the targeted genes from *C. clavus*

Sl. No	Phytochemicals of coconut	Targeted genes from *C. clavus*	PDB No of the Genes
1	lauric acid	TRANSCRIPTION	6EU3
2	myristic acid	RIBOSOME	6EM1
3	linolenic acid	TRANSCRIPTION	5N9J

Molecular Docking
The anti-inflammatory activity of all the phytochemicals reported from Coconut oil was assessed by docking these compounds against the respective active sites of the target proteins. Discovery studio 4.0 was used in this study to find the interacting compounds of Coconut oil with the selected targets of antimicrobials activity by docking **(**Lengauer and Rarey 1996). Docking has found to be the most significant *in silico* analysis for the drug discovery and the analysis of the interaction of the protein or gene molecule (Ciemny *et al*., 2018; Kitchen *et al*., 2004). Strategies of Discovery Studio 4.0 are to exhaustively dock or score possible

positions of each ligand in the binding site of the proteins. Docking study of the target proteins was done with natural compounds derived from Coconut oil to find the preferred orientation and binding affinity of the compounds with each target protein using scoring functions. A molecular dynamics (MD) simulated-annealing-based algorithm, namely, CDOCKER was used to score the interacting compounds. This method uses a gridbased representation of the protein-ligand potential interactions to calculate the binding affinity (Wu *et al*., 2003). CDOCKER uses soft-core potentials, which are found to be effective in the generation of several random conformations of small organics and macromolecules inside the active site of the target protein. Ligands were docked to the proteins followed by scoring them for their relative strength of interaction to identify candidates for drug development. The final poses were then scored based on the total docking energy, which is composed of intra-molecular energy of ligand and the ligand-protein interaction. The lowest energy structure was taken as the best fit. Interpretation of the values was done using standards provided by Discovery Studio such as CDOCKER energy, CDOCKER interaction energy, hydrogen bonds, binding energy etc.

3. Result and Discussion

3.1 Stability of the Oil by UV-Vis Spectrophotometer

The oil formulated in our laboratory showed stability in lab condition as well as when exposed to sunlight. The consistency of the oil in lab condition and sunlight was measured by UV-Vis spectrophotometer. After the incubation period of 24 hrs and 48 hrs in both the conditions there were no significant changes were monitored in UV-Vis. Fig 1 has been showing the results of the spectroscopic analysis.

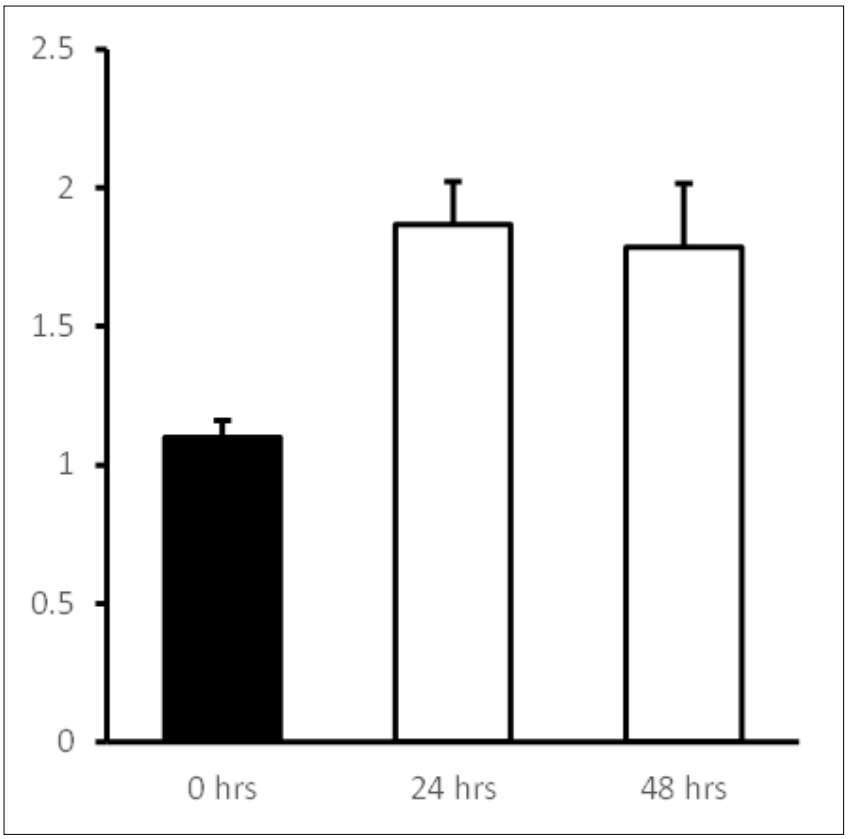

Fig 1: UV-Vis analysis after incubation period of 24 hrs and 48 hrs.

The anti-microbial properties of the oil have been studied against both the bacteria and fungi which have shown a significant result. Coconut oil mixed with ginger oil and vitamin E have shown good results in comparison to the known drugs which is a positive indication of using these oils as a potential antimicrobial drugs which can be used as ointment and skin care substances. We also checked their activity on the fungal growth and these oil have also shown a great effect on reducing the fungal growth thus it can also be used for applying on skin for any fungal contamination. The results of the zone of inhibition in bacteria, fungi and the antibiotics have shown in table 3 and table 4. Fig 2 (a,b,c and d) and (e,f,g and h) are showing the cup disc results of bacteria and fungi respectively.

3.2 Anti-Microbial Property of the Oil

The oil was found effective against the bacteria *Staphylococcus aureus* and the fungus *E. floccosum. S. aureus* infection is common in people with frequent skin injury, particularly if the skin is dry. Skin infections due to Staph are most commonly found in pre-pubertal children and certain occupational groups such as healthcare workers. But it should be noted that they may also cause infection for no obvious reason in healthy individuals. *Epidermophyton floccosum* is a filamentous fungus that causes skin and nail infections in humans. This anthropophilic dermatophyte can lead to diseases such as tinea pedis (athlete's foot), tinea cruris, tinea corporis and onychomycosis. The oil we formulated was found effective against both the bacterial and viral species.

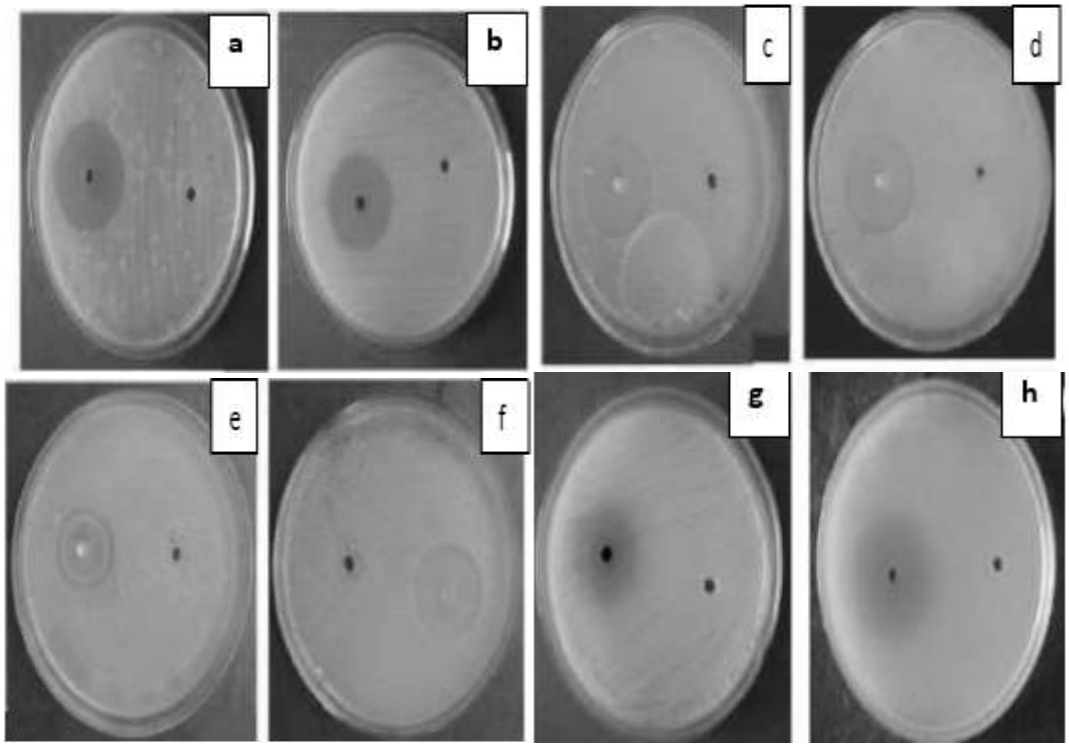

Fig 2: (a) and (b) Bacterial colony growth after 12 hrs and 400X and 500X respectively (c) and (d) Bacterial colony growth after 24 hrs and 400X and 500X respectively (e) and (f) Fungal colony growth after 12 hrs and 400X and 500X respectively (g) and (h) Fungal colony growth after 24 hrs and 400X and 500X respectively

Table 3: Zone of Inhibition of Bacteria: (Values are mean +/- SD of three parallel measurements = Number of zones of inhibition.) (Concentration in ml and Zone of inhibition in mm)

Sl.No	Time	Coconut oil+ginger oil		Coconut oil+gingeroil+vit E			Coconut oil+ginger oil	Coconut oil+gingeroil+vit E		Ampicillin
		(Lab Condition)					(Sun Light)			
		400X	500X	400X	500X	400X	500X	400X	500X	
1	12 hrs	14	7	13	8	16	13	15	10	12
2	24 hrs	16	11	15	10	19	17	17	12	16
3	48 hrs	19	14	17	13	20	20	19	16	20

Table 4: Zone of Inhibition of Fungi: (Values are mean +/- SD of three parallel measurements = Number of zones of inhibition.) (Concentration in ml and Zone of inhibition in mm)

Sl.No	Time	Coconut oil+ginger oil		Coconut oil+gingeroil+vit E			Coconut oil+ginger oil	Coconut oil+gingeroil+vit E		Ampicillin
		(Lab Condition)					(Sun Light)			
		400X	500X	400X	500X	400X	500X	400X	500X	
1	12 hrs	17	17	15	16	19	18	17	19	15
2	24 hrs	19	18	17	18	20	19	18	18	18
3	48 hrs	20	20	18	19	21	22	19	20	22

3.3 In-silico Analysis of the Oil

The ligand molecules with least binding energy are considered as compounds with highest binding affinity. This binding affinity indicated a focused interaction between the above compounds mentioned in Table 1 and 2 with the targets compared to others. The parameters for finding the best inhibitors such as CDOCKER energy, CDOCKER interaction energy and number of hydrogen bonds were also evaluated. CDOCKER energy is the combined energy produced by the sum of internal ligand strain energy and receptor-ligand interaction energy where, CDOCKER Interaction energy is the interaction energy between the protein and ligand and the values of these two parameters indicate the strength of interaction between the proteins and the ligands. Besides least binding energy, compounds with least atomic energy difference between CDOCKER energy and CDOCKER interaction energy were analyzed. Based on CDOCKER energy and CDOCKER interaction energy, Fig 3 is showing the active sites of the interaction of the ligand and the proteins which shows that there was a high binding affinity with both the molecules hence it was shown no significant changes due to the exposure in sunlight.

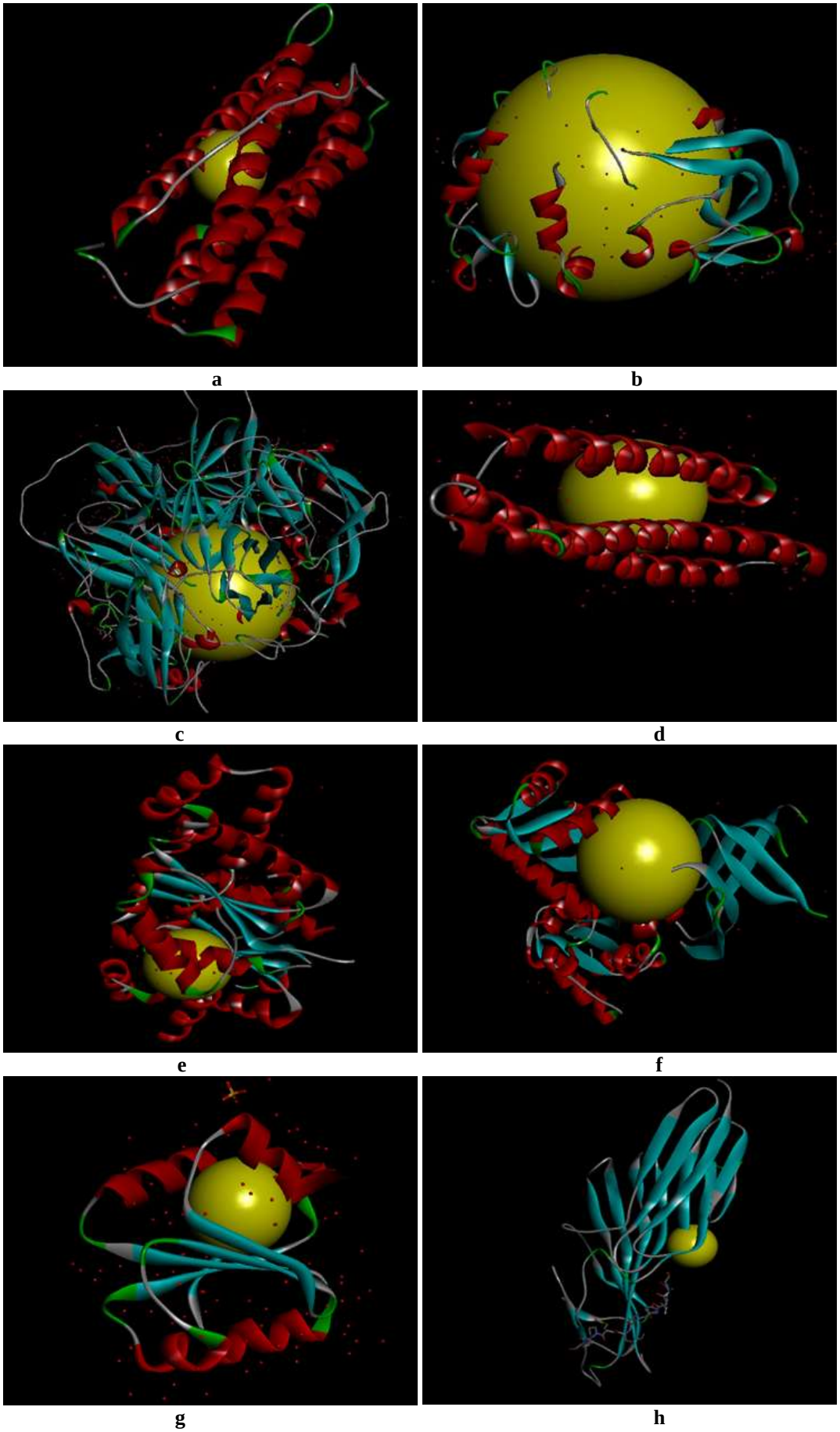

Fig 3: (a), (b), (c), (d) are showing the interaction of the phytochemicals of coconut oil and the bacterial genes. (e), (f), (g), (h) are showing the interaction of the phytochemicals of coconut oil and the fungal genes.

4. Conclusion

The oil described in this article needs more extensive pre-clinical trials with diverse variety of microbes that can cause skin infection. Research is going on to isolate and identify specific phytochemical from ginger and virgin coconut oil responsible for anti-microbial property of the oil. Regular use of this oil will prevent many skin infection especially for people who are working in wild environment and prone to skin diseases. Moreover, vitamin E is a value added component in the oil which is having antioxidant activity, thereby preventing premature aging.

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