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Comparative Antioxidant and Anti-Fungal (Malassezia furfur) Activities of Glycine max L., Vigna radiata L., and Phaseolus vulgaris L.: Optimal Active for Anti-Irritation Efficacy and Nanoemulsion-Based Formulation Development

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Abstract: *Oil extraction efficiency, bioactive properties and formulary potential of soybean (Glycine max L.), green bean (Vigna radiata L.) and red bean (Phaseolus vulgaris L.) oils were evaluated in the present investigation. The oil yields of the hexane extracts were 20.10±0.55%, 2.36±0.32% and 1.92±0.04%, respectively. In general, green bean oil (GBO) had the highest antioxidant potential (lowest IC_{50} values) in DPPH radical scavenging assay (0.62±0.03mg/mL) and lipid peroxidation (0.05±0.01mg/mL, the same as ascorbic acid), compared to other above extracts. Soybean oil exhibited the weakest antioxidant activity, with IC_{50} levels of 0.99±0.05 and 0.59±0.25 mg/mL, respectively. None of the oils tested had a high inhibition against Malassezia furfur compared to that by the positive control, which is ketoconazole (31 mm). HET-CAM test revealed non-irritant activity of green bean oil (irritation score; 0.00±0.00). In vitro formulations screening Nano emulsion formulation (oil green bean; oil/surfactant ratio=1:1) as per preparation technique was found to be most stable in mean of droplet size (110-150 nm) with high zeta potential (-35 mV). These findings suggest that GBO is a well preserved potent natural antioxidant that is non-irritative and can be used in topical formulations.*

Keywords: *Bean oil Antioxidant activities Nano emulsion HET CAM*

INTRODUCTION

Nano emulsions are essentially a colloid in which water and oil components, two normally immiscible liquids, are blended into a single phase. One of the liquids is dispersed into nanometer-sized droplets and the other one is introduced as a continuous medium measuring between 20 and 200 nm in diameter with broadest definitions assuming a size up to 500 nm, and even up to > 1,000 nm depending on the formulation and application [1-3]. For these systems, surfactants are used to stabilize them and the size of the nano emulsions in the form of droplets is very small and makes them instable, transparent, and bioavailable when compared to normal emulsions [3]. Emulsions may be O/W, W/O, or another form of emulsion such as W/O/W etc. Nano emulsions are one such emulsion which consist droplets of small size [3]. Nano emulsions are classified into high-energy type and low-energy type. Among the high-energy methods, ultrasonication is the most used technique, and for low-energy methods phase inversion temperature (PIT) and spontaneous emulsification are very popular. Recent advanced studies reveal that the traditional ultrasonic systems may provide nano emulsions of monodisperse droplet size distribution with reasonable stability that are appropriate for industrial scale-up and long shelf life [4-5]. The present status of nano emulsion research with special focus on preparation methods and application for the (2019–2025) time period [6] and comparative research on the preparation method [5]. Red bean highly pigmented red beans (Vigna angularis or Phaseolus vulgaris, depending on the variety) have 8 grams of protein and 6.5 grams fiber along with plenty of magnesium and phosphorus and potassium and copper and manganese and folate per half-cup cooked. Phenolic, and cyanidin-3-O-glucoside are identified as the antioxidant activity of red bean extracts which improved the oxidative stress and decreased pro-

inflammatory markers in macrophage that has anti-inflammatory effect In vitro [7]. The antimicrobial activities of red bean proteins (lectins and globulins) display activity against pathogenic bacteria and fungi, as reported by [8]. Soybean (Glycine max) can be high in nutrient levels, with 29 g protein (such that at 10 g or 22% of a Daily Value in a 2,095 kJ meal) 10 g fiber (defatted soybean meal), but 15 g fat (with the saturated fat = 29%, polyunsaturated fat = 30% and monounsaturated fat = 41%; no cholesterol), nor vitamins and minerals in significant amount [9]. Mostly it's polyphenols and isoflavones that make soybeans so potent at battling free radicals. Fermentation also elevates the content of the aglycone isoflavones and enhances the antioxidant ability [10]. Soybeans are rich in phenolic compounds that too can kill fungi. Phenolics in general have been reported to be effective in suppressing aflatoxin producing fungi and certain phenolics (e.g., syringic acid and p-coumaric acid) have been related to antifungal activities. mung beans, a common food in Asia. They are also high in protein (49 grams per cup raw), fiber, folate, magnesium and potassium. Its antioxidant activity is attributed mainly to flavonoids, including vitexin and isovitexin. Such compounds are accumulated in higher amounts in the seed coat and are more efficient as scavengers of free radicals than vitamin C and green tea [11-12]. Extracts of green bean sprouts were highly effective as antifungals versus human pathogens (Trichophyton rubrum) and tomato pathogen (Trichoderma harzianum) probably due to increased polyphenolic concentration upon germination [13]. Even with that data, there still isn't enough research on bean oils. The new application is a novel use of antifungal and antioxidant compounds that are fat-soluble and are found in oils from Thai food store cultivars. The word on the street is that nano emulsions stabilize and make things more bioavailable, so they might be the best mode for delivering these compounds. We'll press oils from red, soy and green beans. Thereafter, their DPPH/NO radical-scavenging potential, inhibition of lipid peroxidation, and antifungal action against Malassezia furfur will be assessed. Finally, we will form them into nano emulsions that might have applications in the future.

MATERIALS AND METHODS

Materials

In the current study, the chemicals of ascorbic acid (APS, Brisbane, Australia), dimethyl sulfoxide (DMSO) (Merck, Germany), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and quercetin (Sigma, Germany) (ferrous sulphate (Lobachemie, India) to prepare the standard curve of quercetin), Folin-Ciocalteu's reagent, potassium chloride, sodium acetate and sodium carbonate (Carlo Erba, Germany) were used. Sodium nitrite was purchased from Univar, Austria. Malassezia furfur from the Faculty of Medicine, Chiang Mai University, Thailand and a standard drug, ketoconazole from S. Charoen Pharmacy Trading Co., Ltd., Thailand, were the fungal strain used in this study. Tween 80 was obtained from Chemipan, Thailand. Red bean, soybean, and green bean materials were purchased from Khlong Toei Market, Thailand. Also, hexane, tween 80 and span 80 were purchase from chemipan venders in Thailand. Instruments: Instruments used were a microplate reader (Versa max, USA); 6 mm filter paper discs (Macherey-Nagel GmbH & Co. KG, Germany); Petri dishes (Union Science Co., Ltd., Chiang Mai, Thailand); Laminar Flow Biohazard Class II cabinet (Renovation Technology Ltd. Partnership, Chiang Mai, Thailand); soft incubator SLI-600ND (EYELA, Tokyo Rikakikai Co., Ltd. Japan); rotary vacuum evaporator (BUCHI R300, Switzerland); scanning electron microscope (SEM) (FEI, QUANTA 450, USA); binocular microscope (OPTIKA, SFX-51, Italy); a gas chromatography-mass spectrometry system (GC-MS) (Shimazu QP5050A, Japan); a high-pressure homogenizer (HHP) (Sonics, USA); and nanoparticle size analyzer (Horiba, Japan).

Methods

Extraction of oil from beans

Beans—red beans (Phaseolus vulgaris L.), soybeans (Glycine max L.) and green beans (Vigna radiata L.) were purchased from the Khlong Toei Market, Bangkok, Thailand in December 2025. All of the beans were washed with distilled water, dried at 50°C for 3 days, and powdered in a mechanical grinder, and then these samples were extracted with 5 L of hexane by maceration at room temperature for 7 days. The filtered extracts were concentrated using a rotary evaporator under vacuum to obtain the crude hexane extracts. To calculate the percent yield of crude extract, we used the formula below:

$$\text{Percent Yield} = \frac{\text{Actual Yield}}{\text{Theoretical Yield}} \times 100\% \quad (1)$$

Where: Actual Yield: Weight of the product you obtained in the experiment (g). Theoretical Yield - the most product that can possibly be produced from a given reactant mass based on the balanced chemical equation. The crude extracts collected were kept at 4 °C until analysis and bioactivity test.

Antioxidant activity

The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay is frequently employed to know about the efficacy of an antioxidant [14]. In this way, we use vitamin C (L-ascorbic acid) as the reference. The principle is taken on the basis that when antioxidants are added to a solution of DPPH (0.05 mM) radicals in methanol, they decolourise the purple DPPH radicals to the yellow diphenylpicrylhydrazine. The experimental method is mixing 0.2 ml of the test sample (5.0 mg/ml working concentration) or the standard preparation (0.02 mg/ml) with 1.8 ml of DPPH solution followed by a vigorous vortex and, after 30 min incubation in the dark at room temperature. For each sample, the absorbance (517 nm) is measured three times using a UV-Vis spectrophotometer. To express the percentage of inhibition of radicals, the following equation is obtained:

$$\% \text{ DPPH radical inhibition} = [(A - B)/A] \times 100 \quad (2)$$

where B is the absorbance of the sample DPPH mixture and A the absorbance of DPPH control (absent test compounds). This colorimetric method measures the capacity of a material to neutralize free radicals by donating an electron.

Nitric oxide (NO) radical scavenging activity and inhibition of lipid peroxidation of unsaturated fatty acids

The NO radical scavenging activity and the inhibitory effect on the oxidation of unsaturated fatty acids of the red bean, green bean and soybean oils were investigated. Test samples dissolved in DMSO (0.001-10 mg/ml) were run against two assays: 1) The Griess reagent system were utilized to assay for the scavenging activity. Sulfanilamide and naphthylethylenediamine dihydrochloride (NED) react with nitrite ions (released from the oxidation of NO) to generate a pink chromophore that was read at 546 nm in a spectrophotometer in this system. The activity of lipid peroxidation inhibition was tested according to Wang [15]. Into a test tube were added 0.2 mL at 150 ratio and 0.1 mL of the extract (1 mg/mL), 0.2 mL of ferric salt oxide ($FeSO_4 \cdot 7H_2O$) 25 mM and 1.5 mL PBS pH=7.45. The sample extract was substituted by PBS in the control setting. Lipid peroxidation was determined by adding an equal volume of 0.5% thiobarbituric acid (TBA) in 20% TCA to the reaction mixture. Incubation was as before, but at 95°C for 30 min followed by quenching of the reaction with an ice bath. The samples were then centrifuged at 10,000×g for 30 min, and the resulting supernatants were read at A532. Inhibitory activity on lipid peroxidation – The activity was calculated as % inhibition of lipid peroxidation.

Antifungal activity assay against Malassezia furfur

The antifungal activity was determined using disc diffusion assay against Malassezia furfur. Test oils (red, green and soybean) were filtrated with a 0.2 μm membrane to remove impurities. We made them at 10, 100, and 1,000 mg/ml. The inoculum of M. furfur was prepared at 0.5 McFarland scale (approximately $1 \sim 5 \times 10^6$ cfu/ml) in SD broth as a vehicle with the addition of Tween 80 and distributing uniform with all the agar plates. Similarly, 10 μl volume of each of different oil concentrations was aseptically applied (using 6-mm filter paper triturates discs) on the above-mentioned inoculated agar. Positive control was treated with Ketoconazole (0.2 mg/ml) and negative control was treated with Tween 80 separately. The incubation temperature was 35±1 °C and the incubation time was 3-5 days. These inhibition zones were then measured to the nearest millimeter as diameters of clear zones (clear zones around the discs) for evaluation of antifungal activity [16].

Hen's Egg Test Choroiallantoic Membrane (HET CAM)

The oil to be tested was selected based on its strong antifungal and antioxidant potential revealed in previous studies, as previously reported (HET-CAM assay). During the experiment, sample solutions were directly administered to the CAM (chorioallantoic membrane), and any irritative responses were recorded for 5 min, which is a common time period as per Somwongin [17] and Steiling [18]. The irritation score (IS) was calculated using the formula:

$$IS = [(301 - t_h) \times 5 + (301 - t_l) \times 7 + (301 - tc) \times 9] / 300 \quad (3)$$

where: t_h = first observation time (seconds) of vascular hemorrhage, t_l = first observation time of vascular lysis and tc = first observation time of vascular coagulation. Scores were categorized as follows: 0.0-0.9 indicated no irritation, 1.0-4.9 indicated slight irritation, 5.0-8.9 indicated moderate irritation, and 9.0–21.0 indicated severe irritation, as described by Somwongin [19]. IPM served as a vehicle-control here due to its capacity to dissolve the test material obtained from bean oil. The positive control of the assay was 1% w/v SLS and normal saline as the negative control [19].

Nanoemulsion

Nanoemulsion preparation method

According to Devangshi [20], Tween 80 (T80; HLB 15) and Span 80 (S80; HLB 4.3) reach a cumulative hydrophilic–lipophilic balance (HLB) value of 10 and they are the best surfactant pair for the preparation of oil-in-water (O/W) nanoemulsions. Mixing At the given mixing ratio shown in Table 1, the bean oil is mixed with a T80/S80 solution to obtain a crude emulsion, the bean oil is completely dissolved before mixing. The primary emulsion is treated to decrease the size of the droplets to the nano level.

Table 1: Compositions of nanoemulsions with different oil contents [20].

No.	Oil (% w/w)	Surfactant (% w/w)		Total	Water (% w/w)	Oil:Surfactant Ratio
		Tween 80	Span 80			
1	5	2.7	2.3	5	90	1:1
2	10	2.7	2.3	5	85	2:1
3	15	2.7	2.3	5	80	3:1

The emulsion was then homogenized using a high-pressure homogenizer and sonicated for a further 10 minutes. An oil-water interface was observed at the bottom of the mixture which corresponded with a volume of the separating rest water no longer containing any oil. To completely screw up this interface, the sonication probe was placed directly in the center of the container. An ice-water bath was used to keep the temperature at 28–30 °C, ensuring that the temperature does not increase during sonication. As can be seen from the Table 1, the final emulsions formulation consists of 5% (w/w) surfactants (the combination of Tween 80 and Span 80 with HLB10) and 5–15% (w/w) oil. All the emulsion samples were stored in individual bottles in the dark at room temperature until re-analysis. Seven replicate samples were also synthesized to check valence states and to confirm the measured properties.

Emulsion characterization analysis

Measurement of emulsion droplet size and distribution

The size distribution and intensity profile of emulsion droplet were measured by the nanoparticle size analyzer with the DLS method. All samples were diluted in distilled water by a factor of 1000 prior to measurement to prevent possible multiple light scattering. The refraction indices of the oil and water phase were 1.54 and 1.33 respectively. The Z—average value (average droplet size) was recorded in each instance. The samples were run in duplicates with each duplicate being performed in triplicates to enhance the reproducibility and the reliability of the results [20].

Morphological characteristics

Morphologies of emulsions were observed by a scanning electron microscope (SEM) under a magnification of ×50,000. A tiny droplet of the emulsion sample surface was carefully placed on a clean glass slide and carefully spread out with a cover glass, without producing air bubbles. All analysis was performed at room temperature. To provide for thorough morphology evaluation, a photo was needed from more than one section of each sample. All of the SEM images were collected with a computer interfaced with the microscope and commercial digital image processing software.

Statistical analysis

Data are shown as the mean ± SD of at least three independent experiments. The data among groups were compared with one-way ANOVA and the Tukey's post hoc test was used to find the statistically significant differences at $p < 0.05$. GraphPad Prism version 10 was used for all statistical analyses.

RESULTS

Extraction of oil from beans

Oil extraction was performed using maceration method with hexane as the solvent from the seeds of three types of beans: soybean (Glycine max L.), green bean (Vigna radiata L.) and red bean (Phaseolus vulgaris L.). By comparing the physical properties of the extracted oils, it was found that hexane extraction brought oils with significantly different appearances (particularly with color, as in Figure 1. The green beans oil obtained were pale yellow in color, the red beans oil were yellow in color, and the soybeans oil had the least yellow color. Characteristics Hexane is a colorless volatile solvent with a boiling point of 69°C. It is very easy to separate from oil without bleaching the color or quality of the oil. Hexane is an also an excellent solvent for vegetal oils, it preferentially removes the oil, proteins, sugars, and undesirable gum are left in the bean kernels. Extraction yield was significantly different for the different types of beans. The yields of oils of soybean, green bean and red bean were 20.10 ± 0.55%, 2.36 ± 0.32% and 1.92 ± 0.04%, respectively (Table 2). Santos et al. (2013) recorded 10.36% of soybean oil yield with hexane. The comparison of oil extracted from seed showed that Jatropha curcas L. was the seed with the highest oil

content at 48.57%, followed by Arachis hypogaea L. at 43.07%. For all seeds tested hexane showed better results compared to ethanol. Hexane also extracted more oil than ethyl acetate and ether. The variation in yield is a function of hexane's varying ability to penetrate oil-storing cells and its ability to dissolve oils in different categories of beans. The variations between the colors and compositions of the oil are because of the varying content of vital substances in the types of beans, he explains. Soybean is the best source of protein content which ranges from 36.56% to 39.70%. The second most protein and red bean the third most protein (25.99–26.56% and 22.84–25.22%, respectively). Green beans are rich in phenolic compounds and flavonoids, which are so important for color and quality of oil. For instance, vitexin and isovitexin are responsible for the oil yellow light color [21]. Red beans are rich sources of anthocyanins and proanthocyanidins and it is these compounds that make it red and very antioxidant [21]. Soybean has the fattest, which is 22.02%, green bean and red bean are less fat. This can be understood as a reason for the difference in the oil yield and the color [22]. These variations are due to genetics, growing environment and the specific chemical composition of each bean specie.

Table 2: Comparison of mean percent yields of oils from different bean species

Sample	Mean percent yield of oil (Mean ± S.D.)
Glycine max L.	20.10 ± 0.55^{a}
Vigna radiata L.	2.36 ± 0.32^{b}
Phaseolus vulgaris L.	1.92 ± 0.04^{b}

Note: Different superscript letters (a, b) indicate statistically significant differences ($p < 0.05$) among the mean values.

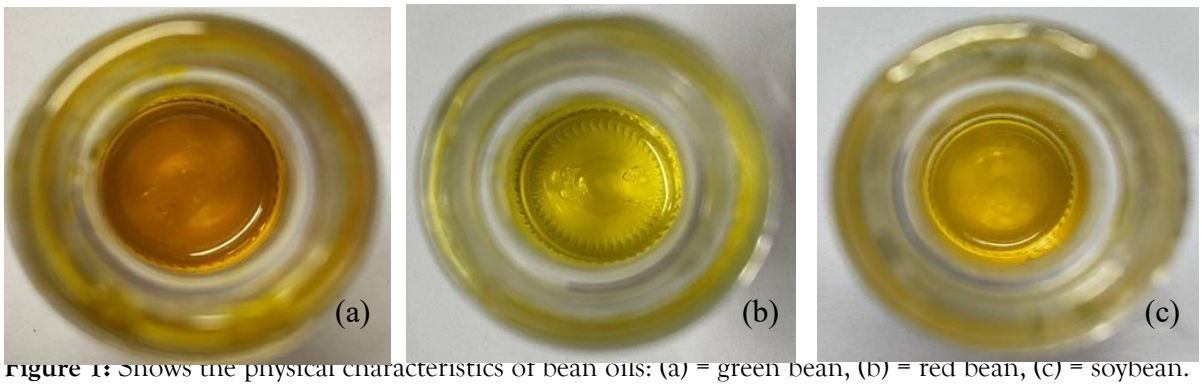

Figure 1: Shows the physical characteristics of bean oils: (a) = green bean, (b) = red bean, (c) = soybean.

Antioxidant activity

Antioxidant activity by DPPH radical scavenging method

The IC_{50} for the Vigna radiata L. oil (green bean) and Phaseolus vulgaris L. oil (red bean) in the DPPH radical scavenging test were determined to be 0.62 ± 0.03 mg/ml and 0.67 ± 0.12 mg/ml, respectively. The significance of difference of test results were remarkably different from that of soybean oil (Glycine max L) whose the value was 0.99 ± 0.05 mg/ml ($p<0.05$). This means the first two oils did a better job getting free radicals out of the body. Antioxidant activity in the DPPH test is measured by the ability of an antioxidant to change the color of a purple DPPH (1, 1-Diphenyl-2-picrylhydrazyl) solution to yellow color. Antioxidants are electron and/or hydrogen atom donor showing free radical scavenging capacity [23]. The smaller the IC_{50} value the better it is as an antioxidant, because it puts that a substance is able to inhibite better of radicals free such as DPPH. This finding is consistent with the findings of other studies conducted with phenolic and flavonoid compounds, which have been reported to be the most effective compounds in neutralizing DPPH radical, and total antioxidant activity rate was higher in beans [24].

Antioxidant activity by Nitric Oxide (NO) method

The antioxidant potentials of oils extracted from three beans, namely Vigna radiata L. (green bean), Phaseolus vulgaris L. (red bean) and Glycine max L. (soybean), were assessed by using nitric oxide radical scavenging assay in the present investigation. The NA results did not relate to any of the three bean oils. Nitric oxide radicals were scavenged by the oils in a concentration-dependent fashion. NO is indispensable for many physiological functions, including smooth muscle relaxation and suppression of platelet aggregation and transmission of neurotransmitters in neurons [24-25]. Excessive nitric oxide can be lethal to cells and tissues. In view of this scavenging of nitric oxide by these bean oils, possible medical applications are indicated. It has been reported that plant extracts abundant with phenolic compounds might yield nitric oxide scavenging activity because of hydrogen transfer, or metal-catalyzed reaction between the active moiety and NO radical [26].

Antioxidant activity of unsaturated fats method

The oil from Vigna radiata L. (green beans) was found to possess the highest lipid-peroxidation-inhibiting activity in the test for antioxidant activity against unsaturated fat oxidation (Table 3). Its IC_{50} did not significantly differ from that of ascorbic acid (0.04 ± 0.00 mg/ml). Glycine max L. showed low activity (IC_{50} 0.59 ± 0.25 mg/ml) compared to P. vulgaris L. (IC_{50} 0.07 ± 0.01 mg/ml) oil. Lipid peroxyl radicals initiate a free radical chain reaction leading to lipid oxidation and cytotoxic intermediates [27]. Antioxidants inhibit this by binding metal ions or donating hydrogen to the lipid radicals. The high amounts of lipophilic compounds (phenolics) in green bean oil may be responsible for its impressive potency, as it would stimulates antioxidant activity in lipid systems [28].

Correlation of antioxidant activity

Similarity among antioxidant assay correlation of antioxidant activity between the three assay methods is shown in Table 4. Vigna radiata L. (green bean) had a high capacity of lipid peroxidation inhibition (IC_{50} = 0.05 mg/ml) and a radical scavenging capacity activity (IC_{50} = 0.62 mg/ml). Phaseolus vulgaris L. (red bean) had similar effects, although they were not as pronounced. This correlation implies that antioxidant compounds in beans play a role by inhibition of not only water-soluble radical (DPPH) but also lipid oxidation [29]. In all assays, Glycine max L. (soybean) was the least effective, which ostensibly could be attributed to differences in antioxidative constituent composition, and particularly the type and quantity of phenolic compounds [27]. On the basis of these experimental observations in regard to the antioxidant activity of oils from Vigna radiata L. (green bean), Phaseolus vulgaris L. (red bean), and Glycine max L. (soybean), tested by DPPH radical scavenging method, NO assay, and lipid peroxidation inhibition, it can be inferred that Vigna radiata L. (green bean) oil demonstrate the most potent antioxidant activity overall and in lipid peroxidation inhibition among the tested oils, even similar to ascorbic acid (serving as control). Next came the red bean (Phaseolus vulgaris L.) and soybean (Glycine max L.). The variations are likely a result of the distinct types and levels of bioactive compounds found in each variety of bean.

Table 3: Comparison of antioxidant activity

Sample	DPPH Radical Scavenging Activity IC_{50} (mg/ml) (Mean ± S.D.)	Nitric Oxide (NO) Radical Scavenging Activity SC_{50} (mg/ml) (Mean ± S.D.)	Lipid Peroxidation Inhibition Activity IPC_{50} (mg/ml) (Mean ± S.D.)
Glycine max L.	0.99 ± 0.05^{d}	NA	0.59 ± 0.25^{c}
Vigna radiata L.	0.62 ± 0.03^{b}	NA	0.05 ± 0.01^{a}
Phaseolus vulgaris L.	0.67 ± 0.12^{c}	NA	0.07 ± 0.01^{b}
Ascorbic acid	0.04 ± 0.01^{a}	NA	0.04 ± 0.00^{a}

Note: Different superscript letters indicate statistically significant differences ($p < 0.05$); NA = No activity detected

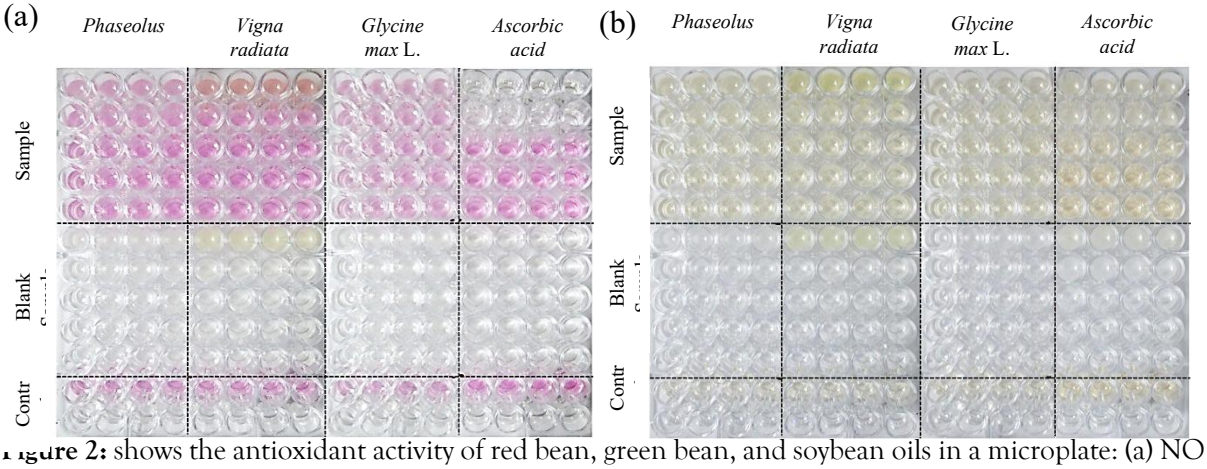

Figure 2: shows the antioxidant activity of red bean, green bean, and soybean oils in a microplate: (a) NO radical scavenging activity and (b) inhibition of unsaturated fatty acid oxidation.

Antifungal activity against Malassezia furfur

The antifungal activities of green bean, red bean and soybean oils (at concentrations of 0.1-10 mg/ml) in connection with Malassezia furfur were also measured. The findings showed that no one of these oils has clear inhibition zone against Malassezia furfur (results are presented in the Figure 3., compared to the

ketoconazole 0.2 mg had an impressive average zone of inhibition 31 mm. Ketoconazole adopted as positive control showed a significant antifungal activity against strain. Previous reports indicated that ketoconazole has potential strong antifungal activity against Malassezia furfur, with a minimum inhibitory concentration (MIC) of 0.1 ± 0.1 µg/ml [30] and can suppress the morphological transition of the fungus from yeast to pathogenic mycelium. Ketoconazole is also frequently used as a reference standard compound to assess the antifungal activity of other extracts or natural products on disc diffusion assays [31]. As for extracts from other plants or herbs, for Cassia alata L. [32] and Buni fruit (Antidesma bunius) [31] with the existence of moderate antifungal activity on M. furfur reported depending on extract type and concentration. On the contrary, oils from Vigna radiata L. (green bean), Phaseolus vulgaris L. (red bean) and Glycine max L. (soybean) showed no appreciable inhibition against Malassezia furfur in this study.

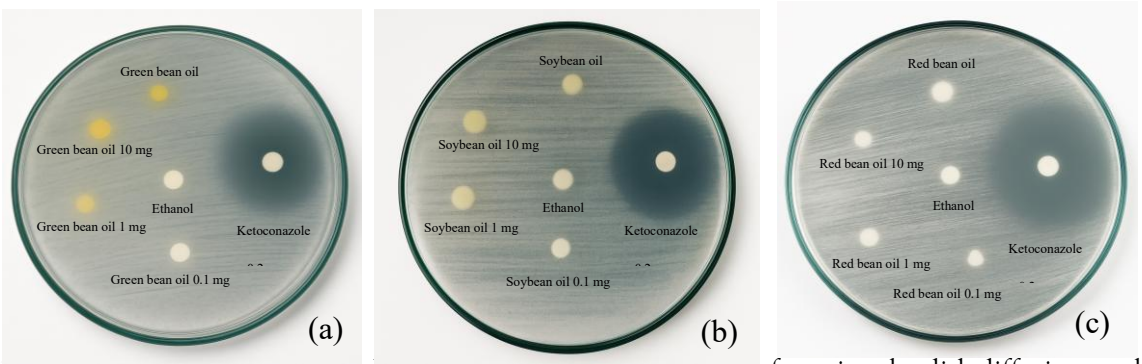

Figure 3: Results of the antifungal activity test against Malassezia furfur using the disk diffusion method, comparing three bean oils – (a) red bean oil, (b) green bean oil, and (c) soybean oil – with the standard drug Ketoconazole.

HET CAM (Hen's Egg Test Chorioallantoic Membrane)

The irritation score (IS) tabulated in Table 4 measures the level of irritation of different samples according to the result of the HET-CAM. The positive control (1% w/v SLS) showed a significant irritation score of 18.32 ± 0.02 that was considered to cause "severe" irritation to the skin. This early observation is in agreement with already existing knowledge about SLS as an irritant and therefore proves that the test is sensitive and reliable.

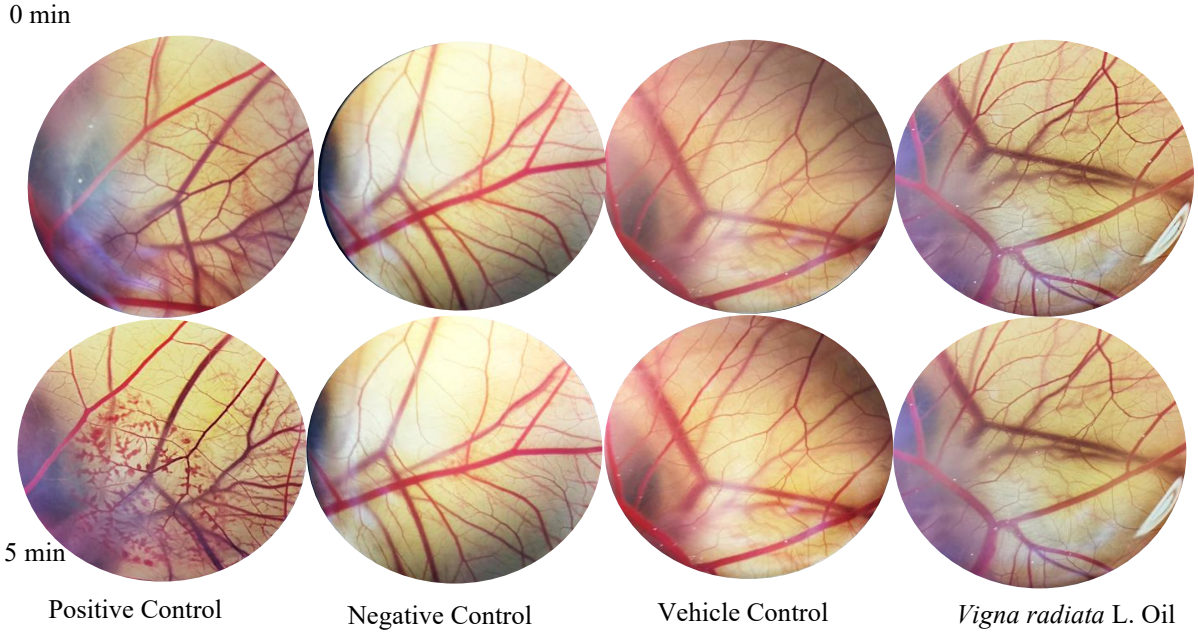

Figure 4: Shows result of Hen's Egg Test Choroiallantoic Membrane (HET CAM**)** test

In contrast, the negative control (0.9% w/v NaCl solution), vehicle control Isopropyl Myristate (IPM), and the test sample (Vigna radiata L. oil, 10 mg/ml) all recorded an irritation score of 0.00 ± 0.00, corresponding to "no irritation." These findings indicate that both the negative control and vehicle control are non-irritant, as expected. More importantly, the Vigna radiata L. oil at the tested concentration did not induce any detectable irritation, suggesting it is safe for topical application under the conditions of this study (Figure 4.).

Table 4: Irritation score of samples

Sample	Irritation score, Mean ± SD	Result
1% w/v Sodium lauryl sulfate (Pos.)	18.32 ± 0.02	Severe
0.9% w/v NaCl solution (Neg.)	0.00 ± 0.00	No irritation
Isopropyl Myristate (Veh.)	0.00 ± 0.00	No irritation
Vigna radiata L. oil (10 mg/ml)	0.00 ± 0.00	No irritation

Note: Pos. = Positive control, Neg. =Negative control, Veh. =Vehicle control

Nanoemulsion

Preparation of nanoemulsion

The nanoemulsion formulation studies also evaluated the use of different oil-to-surfactant ratios and oil types which resulted in significant differences in emulsion stability and particle properties. The analysis of oils from green bean, red bean (Phaseolus vulgaris L.) and soybean (Glycine max L.) showed that the green bean and soybean oils had a better stability of nano emulsions formation, in comparison with the red bean oil, probably due to differences in the composition and physical properties of examined oils. Three oil/surfactant ratios were investigated, at 1:1 (5% oil, 5% surfactant), 1:2 (10% oil, 5% surfactant), and 1:3 (15% oil, 5% surfactant). In the assay from 1:1, the nano emulsions were well-dispersed; however, the increased oil contents (1:2 and 1:3) at the same 5% of surfactant condition decreased the particle stability and particle size distribution with the accordance in Figure 5. Findings by [20] for the influence of oil type on the properties of nano emulsions. Very effective nano emulsification has been found with the Tween 80: Span 80 mixed surfactant system with a surfactant ratio of 2:7:2:3 in surfactant stabilized systems. Another reason why the blend is more effective in reducing the interfacial tension between the oil and water phases may be that Tween 80 with an HLB of 15 and Span 80 with an HLB of 4.3 are relatively hydrophilic and lipophilic, respectively. Formation of nanoparticles**,** the solution of surfactant mixture was warmed to 45–50°C for 30 min to ensure complete dissolution, followed by the homogenization of the mixture under pressure to form nanoparticles which were of uniform size distribution. This technique is also important to stabilize the resulting emulsions, as it allows to control a) the distribution of the particle size b) the homogeneity and the optimum dispersion.

Figure 5: Shows nano emulsions in the ratio between oil (% w/w) and surfactant (Tween 80 + Span 80) (% w/w), where (a) = green bean oil, (b) = soybean oil, and (c) = red bean oil.

Characteristic analysis of nanoemulsions

Measurement of nanoemulsion droplet size and distribution

The particle sizes of the nanoemulsions which were produced from three bean oils, mung bean (Vigna radiata L.), red bean (Phaseolus vulgaris L.), and soybean (Glycine max L.), were 110–165 nm, according to the results of the research. The smallest particles were detected in V. radiata L. (110–150 nm) and G. max L. The visualized large particles were observed in Phaseolus vulgaris L. (130–165 nm), as shown in Figure 6. The particle size also increased slightly with the oil-to-surfactant ratio ranging from 1:1 to 3:1 for all particles. This is in accordance with a report by McClements [33] who showed that with higher oil in formulations, an increase in particle size may occur due to the higher degree of coalescence of oil droplets when a larger dispersed-phase volume is applied [34]. The different particle size of the beans has been attributed to the variation in the chemical composition and physical characteristics of the oils which would influence the oil–surfactant interactions [34].

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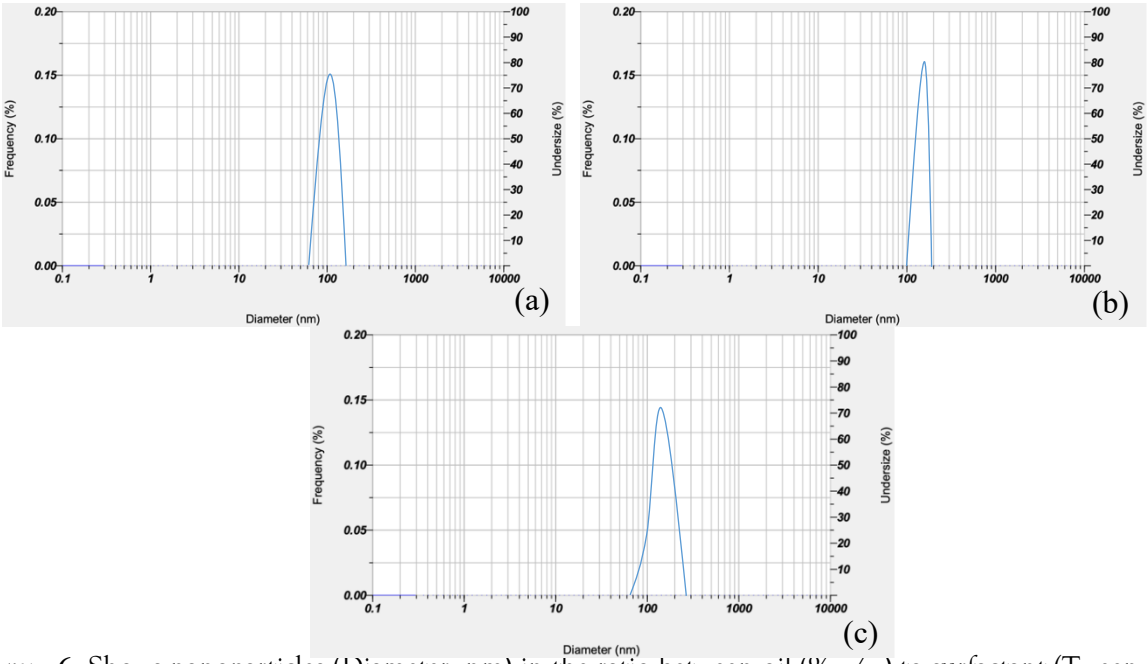

Figure 6: Shows nanoparticles (Diameter, nm) in the ratio between oil (%w/w) to surfactant (Tween 80 + Span 80) (%w/w) (1:1), where (a) = green bean oil, (b) = soybean oil, and (c) = red bean oil.

Measurement of Zeta Potential

Analysis of the zeta potential indicated that nano emulsions prepared with all 3 bean oils displayed negative zeta potential between -25 and -35 mV. Of these, Vigna radiata L. recorded high value (-27 to -35 mV) for the magnitude of -charge and showed a certain colloidal stability according to the results. The observed negative charge is due to the surfactant system Tween 80 and Span 80, which add anionic properties to the surface of oil droplets [21]. In DLVO theory, high zeta potentials larger than ±20 mV contribute to the stability of colloidal systems by increasing electrostatic repulsion between particles of identical charge and inhibit the aggregation and coalescence. Vigna radiata L. exhibited high zeta potential and this could be attributed with the aqueous solubility of the oil components that affects the orientation of surfactant molecules at the oil-water interface and the position of the shear plane [35]. The results of particle size as well as Zeta potential comparison revealed that irradiated Vigna radiata L. The (green bean) oil formulations at 1:1 of oil-to-surfactant ratio showed the best formulation, which had the smallest particle size (around 110 nm) as illustrated in Figure 7. and a perfect Zeta potential (-35 mV) value. These results suggest good emulsion stability as a result of decreased particles size that leads to higher SSA and stability characteristic; and higher zeta potential that prevented particles aggregation due to electrostatic repulsion.

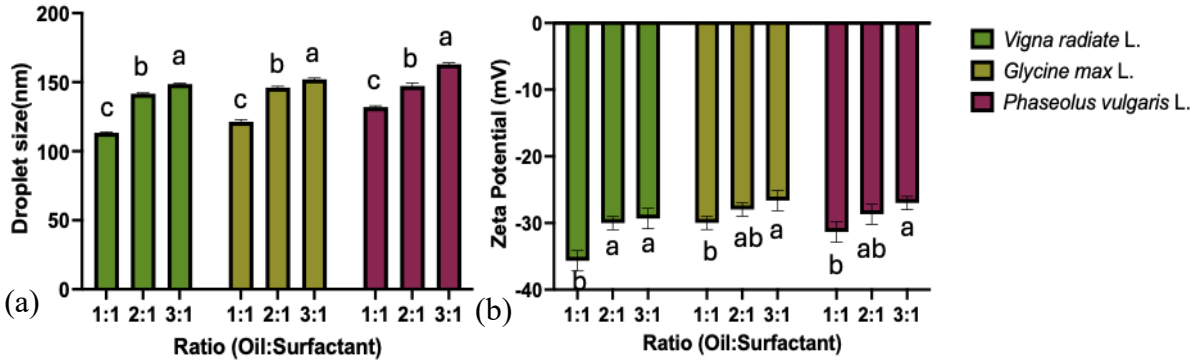

Figure 7: Shows a comparison of Droplet size (nm) (a) and Zeta potential (mV) comparison (b) for each ratio of oil (% w/w) to surfactant (Surfactant; Tween 80 + Span 80) (% w/w) (1:1, 2:1, and 3:1) using Vigna radiata L. (green bean oil), Phaseolus vulgaris L. (red bean oil), and Glycine max L. (soybean oil).

Morphological characteristics

The morphology of the emulsion was observed using scanning electron microscopy (SEM) (magnification, ×50,000). The size of the nano emulsion particles in green bean oil (Vigna radiata L.) was analyzed, and it is shown in Figure 8. The particle size distribution was quite uniform based on spherical well dispersed particles seen in the micrographs. The size distribution in the measured nanoscale signifies that the

emulsion was prepared by Tween 80 and Span 80 surfactant in ratio of 1:1 oil to surfactant, was formulated. This methodology improved emulsion stability as it increased the surface area and overcame the limitation where not all droplets are covered by the surfactant. The decreased particle size decreases the probability of particle aggregation and improves its physical and biological properties, i.e. skin permeation of cosmeceuticals and pharmaceuticals and the bio efficacy of bioactive compounds delivery. A homogeneous dispersion of the particles advantageously eliminates long-term sedimentation and phase separation issues. The SEM data confirm that the used formulation method successfully prepared a stable nano emulsion with the morphological appearance suitable for the functional use.

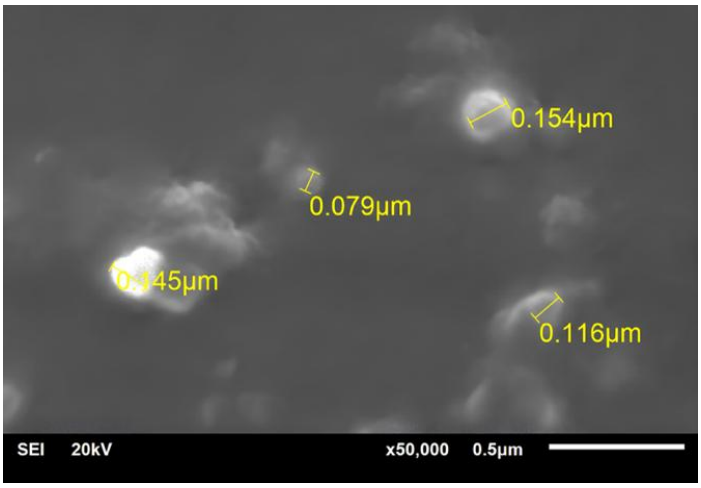

Figure 8: Shows the nanoemulsion particle size of Vigna radiata L. oil in a 1:1 oil-to-surfactant ratio (%w/w) (Surfactant: Tween 80 + Span 80) captured using a scanning electron microscope (SEM) at 50,000× magnification, 20 kV accelerating voltage, and 0.5 µm scale bar.

DISCUSSION

The paper reports detailed comparative analysis of oils from soybean (Glycine max L.), green bean (Vigna radiata L.) and red bean (Phaseolus vulgaris L.) seeds. In the specific case of extraction yield, antioxidative activities, antifungal activity, biocompatibility, and nano emulsion formulation. Onto oil yield and physical properties, extraction by hexane, which is known to be more efficient in non-polar compounds, provided the best one for soybean seeds (20.10±0.55%). The high soybean yields showed the naturally high oil and fat levels present in soybeans when compared to the legumes [36-37]. Meanwhile, the extraction yield of green bean and red bean was relatively low with a value of 2.36 ± 0.32% and 1.92 ± 0.04%, respectively, which indicates the natural variability of constituent composition among various species [38-39]. Moreover, the oils exhibited drastically different colors, pale yellow in green bean oil and golden yellow in red bean oil. This variability may be related to the species-specific number of pigments and antioxidants such as flavonoids, anthocyanins, carotenoids. The advantage of using hexane as a solvent is reflected in higher extraction of pure oils, as well as in the preservation of typical colors and quality of all the samples [40]. The evaluation of antioxidant activity via DPPH radical scavenging, nitric oxide inhibition, and lipid peroxidation assays indicated that green bean oil exhibited the most pronounced antioxidant effects. Green bean oil exhibited the lowest IC_{50} values in both DPPH (0.62±0.03 mg/ml) and lipid peroxidation inhibition assays (0.05 ± 0.01 mg/ml), outperforming red bean oil and significantly exceeding the antioxidant capacity of soybean oil [41-43]. The greater antioxidant action detected may be due to the higher content of phenolic compounds present in green bean oil and mainly in vitexin and isovitexin. On the other hand, the extracted soybean oil presented the lowest radical scavenging capacity, and this was in line with the lowest phenolic content of this sample. These results corroborate with the fact that the structural configuration of bioacidic components in plant oils and their concentration are crucially affecting the antioxidant activity and can be explained based on the literature [42-43]. As regards their antifungal activities, none of the oils examined, although possessing a high antioxidant content, was found to product any gauge worthy inhibitory effects on M. furfur under the test conditions at the disk diffusion test. This result was in stark contrast with the positive control, ketoconazole, that was attributed a large inhibition zone. These results suggest that, at the tested concentrations, their main constituents were insufficient to prevent the growth of Malassezia furfur. This contrasts with the specific reports in literature where some plant extracts and other kinds of natural oils were found to have some moderate antifungal activities [44-46]. For biocompatibility, green bean oil

showed no irritation in the HET-CAM (median irritation score: 0.00) as well as the negative and vehicle controls. In contrast, sodium lauryl sulfate showed marked irritation (positive control). This result reinforces the safety of the oil from green coffee use topically and confirms that it does not presents acute irritant activity at practically used concentrations [47]. Lastly, investigation of the formation and stability of nano emulsions showed that green bean oil and soybean oil were highly potential candidates in the production of a stable nano emulsion especially using an oil/surfactant ratio of 1: 1 in the presence of combination Tween 80 and Span 80 [48]. It is noteworthy that green bean oil produced the smallest and relatively well-dispersed nanoparticles (110–150 nm) with the maximum negative zeta potential (-27 to -35 mV), indicating good colloidal stability [1,49]. The smaller particle size and high zeta potential are crucial for emulsion stability, which is particularly important for uses in such as drug delivery and cosmetic applications. This nano emulsion spherical shape and suitable size range was also confirmed by scanning electron microscopy, which supports the potential of these as delivery systems of functional and bioactive compounds into a variety of applications [50-51].

CONCLUSION

Based on antioxidant properties and nano emulsion characteristics, green bean oil from Vigna radiata L. was the most potential product against the other two bean oils tested. Despite its poor oil extraction rate (for example, 2.36% versus 20.10% for soybean oil), green bean seed oil demonstrated excellent radical scavenging ability and potent lipid peroxidation inhibition that were higher than those of red bean and soybean oils. It is also described its topical application, as confirmed by a HET-CAM value = 0.00, showing the absence of irritancy. The most stable nano emulsions were those prepared using equal oil-to-surfactant ratio, which gave the lowest particle size and highest zeta potential (−35 mV for green bean oil). This indicates that the green bean oil is more suitable to preparation of oxidative stable and high antioxidant level such as nano emulsions which is used for cosmetic or pharmaceutical purposes.

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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

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