International Journal of Progressive Sciences and Technologies (IJPSAT)
ISSN: 2509-0119.

http://ijpsat.ijsht-journals.org
Vol. 20 No. 1 April 2020, pp. 194-200

Physical Properties, Antibacterial and Antioxidant Properties of Raw South Africa Shea Butter against Samples from Libyan Market

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Abstract – Vigorous research has been conducted into the phenology of the shea tree, its usage and that of the shea butter extracted from the nuts of the shea fruit. Shea butter is the most valuable product in the Shea tree and its use as raw or in cosmetic and pharmaceutical products was expanded in the last years. The aim was to carry out comparative study between imported South African raw Shea Butter, one sample from Poland and samples from Libyan market on their physical properties (organoleptic), thin layer chromatography (TLC) chromatogram, phytochemical screening and diphenylpicrylhydrazil (DPPH) scavenging activity. As well as antimicrobial screening. Organoleptic test was carried out by comparing color and odor. TLC chromatogram was performed by spotting solution of samples in hexane on TLC plate and eluted twice in hexane: ethylacetate 8:2 to get good separation. Phytochemical screening was performed to determine the presence of carbohydrates (Fehling test), steroids, and triterpens (Salkwiski test). DPPH assay was carried out by spraying TLC sample spots by 0.2% DPPH methanolic solution. Antimicrobial test was conducted on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Mueller-Hinton agar was used with ciprofolxacin as +ve control and DMSO as–ve control. As a result, Shea Butter samples showed wide diversity in color and odor which considered normal except two white samples that might undergo refining process, while spreadability and TLC chromatograms were similar. Samples and control showed presence of carbohydrates, steroids and triterpens. Control and samples had DPPH scavenging activity. Shea Butter control and samples had no antimicrobial activities against *P. aeruginosa* and *S. aureus*.

***Keyword* – Shea butter, DPPH, Organoleptic, *Pseudomonas aeruginosa*, *Staphylococcus aureus*.**

I. INTRODUCTION

The shea tree (*Vitellaria paradoxa*) is perennial and deciduous and occurs mainly on dry open slopes [1]. The tree has gained importance as an economic tree because of the heavy demand for its butter both locally and internationally.

The shea tree also known as (*Vitellaria paradoxa* or *Butyrospermum parkii*) [2] grows wildly in the dry savannah belt of West Africa, from Senegal, in the west, to Sudan in the east. It was reported that the shea tree produces fruit which has multiple uses at the local level; it is highly nutritious and is also a valuable commodity on the local, national and international markets, making it the ideal candidate to research and invest in [3]. Shea tree is the second most important oil crop in Africa after palm oil [4], but as it grows in areas unsuitable for palm growth, it takes on primary importance in West Africa.

In traditional medicine, Shea butter has been employed in the treatment of several ailments. It encourages wound healing and soothes skin irritation. Shea-butter is also used to treat inflammation, rashes in children, dermatitis, chapping and ulcers, as well as rub for rheumatism [5]. Its leaf decoctions are used for stomachache, headache and as an eye lotion. Roots and root bark are taken orally to cure jaundice and

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chronic sores. They are also used for the treatment of gastric problems as well as diarrhea and dysentery. Bark is used to facilitate childbirth and encourage lactation after delivery. Additionally, it is used as a footbath to neutralize venom of the spitting cobra [6]. Cosmetics use Shea-butter as a result cosmetic industries market uses these ingredients in soaps, shampoo and skin cream preparations [6]. It also has been studied as a potent medicinal plant [7], against bacterial infections [8] and fungal infections [9]. The ethanolic extraction of the active principle of this medicinal plant is more efficacious than water or acetone [8]. It was also showed that potency of a plant extract depends on both the concentration used and the method of extraction [10].

Shea Butter contains high level of ultra violet absorbing triterpenes esters which include cinnamic acid, tocopherols (vitamin A), and phytosterols [11]. Another study confirmed that Shea Butter contains a high level of unsaponifiables (5 - 15%) which include phytosterols (campesterol, stigmasterol, ß-sitosterol and σ spinosterol), Triterpenes such as cinnamic acid ester, σ- and ß-amyrin, parkeol, butyrospermol. Lupeol and a hydrocarbon called karitene [12]. Analysis of the kernel reveals the presence of phenolic compounds such as gallic acid, catechin, epicatechin, epicateachingallate, gallocatechin, epigallocatechin, epigallocatehingallate, as well as quercetin and trans-cinnamic acid [13].

This study aimed to compare physical properties (organoleptic), TLC chromatogram, phytochemical screening, DPPH scavenging activity and antimicrobial screening of imported South African raw shea butter and samples from Libyan market.

II. Materials And Methods

2.1 *Sample collection*

Control samples brought from South Africa (Benin traditional markets). Total of 18 different samples were collected from different sales centers in Tripoli including pharmacies, beauty shops and spices shops. In addition to one sample brought from Poland.

2.2 *Media and Microorganisms*

Mueller-Hinton agar was prepared by adding 23.0 g of media to 1000 mL of demineralized water in a container and sterilized for 20 minutes at 120°C. Media was obtained from Faculty of Pharmacy, University of Tripoli. Well diffusion method was used. Microorganisms (*Pseudomonas aeruginosa* and *Staphylococcus aureus*) were obtained from Faculty of Pharmacy, University of Tripoli.

2.3 *Comparison of physical properties of control and samples*

2.3.1 *Organoleptic tests* (*OLT*)

Shea butter control and samples were tested for their odor and color. samples color was recorded in a grade of shades. Odor was recorded as slight and strong grades.

2.3.2 *Spreadability test*

Shea butter control and samples were spread using spatula on white clean filter paper. Texture, spreadability and presence of small particles were recorded.

2.4 *Thin layer chromatography (TLC) chromatogram*

Shea butter samples and control were dissolved in hexane and spotted on TLC then eluted in 8:2 hexane: ethyl acetate twice until good separation was achieved. TLC plates were checked under UV light.

2.5 *Phytochemical screening test*

The qualitative chemical screening tests were carried out on all samples using standard procedures to identify the presence of reducing sugars, steroids and triterpenoids [14]

2.6 *Antioxidant scavenging activity (DPPH) test*

Samples solutions were spotted on two TLC plates, one was kept the same and the other was eluted. Vitamin C was used as +ve control. TLC plates were sprayed with 0.2% DPPH methanolic solution. Change in color from violet to yellow was considered +ve result.

2.7 *Antimicrobial screening tests*

Well diffusion method on Mueller-Hinton agar was used for this test. Tested bacteria were *P. aeruginosa* and *S. aureus*. Shea butter was suspended in DMSO 1mg/ml. Three concentrations (100, 50 and 25 %) was prepared by serial dilution method. 100 µl of each concentration was added to agar wells. Ciprofloxacin was used as +ve control and DMSO was used as – ve control. Zone of inhibitions were measured after 24 hrs of incubation at 37 C°. Results were compared to ciprofloxacin +ve control.

III. Results And Discussion

3.1 *Organoleptic test (OLT)*

Raw and unrefined Shea Butter has a slightly smoky scent and range in color from off-white to yellowish and even yellowish with a faint green tinge ([15], [16]). All these colors are normal and have no impact on the quality. In refining process of Shea Butter, high temperature is used as well as hexane and other chemicals in order to remove color and odor.

As a result, many of the healing properties of shea butter are lost in this process with retaining of moisturizing qualities. The resulting product will be white and hard and it will lose much of the bioactive compounds that are present in natural Shea Butter [15].

In this study, samples and control showed wide diversity in colors and odors. The colors were ranging between white, ivory, off white, pale yellow and dark yellow color. While odors were ranging between perfumed to unpleased. The two white samples (A10, A12) indicat that they might be refined which might cause the loss of minor but valuable components such as unsaponifiable fraction with medicinal properties [17]. Since all the other samples used in this study were unrefined Shea Butter. Yellow color was observed possibly due to the retention of β-carotene, in that the butters did not undergo refining process which leads to the removal of carotenoids and thus the loss of characteristic color [18]

The fragrance scent of some samples might be due to the presence of some additives that were added to mask rancidity odor to be acceptable by consumers or due to refining processes that possibly take place during production (figure 1).

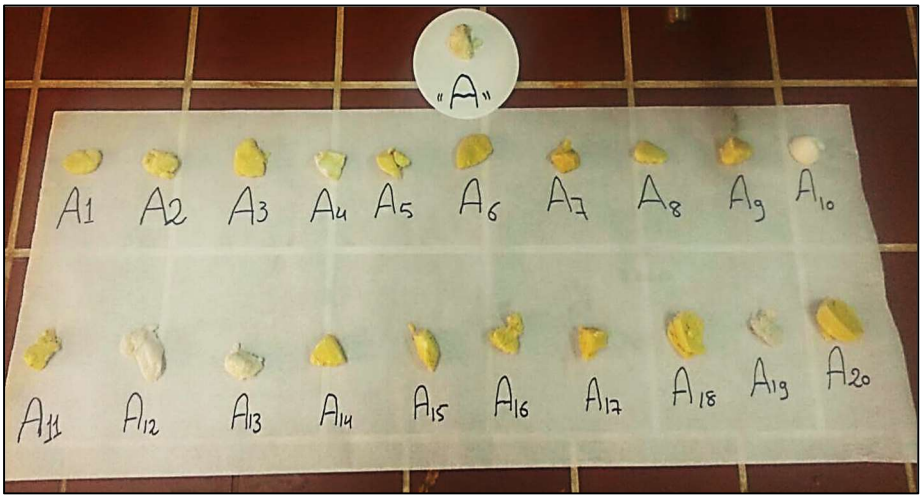

Figure 1: Color of Shea butter control (A) and samples (A1-A20).

3.2 *Spreadability test*

Raw unrefined shea butter is soft and creamy [15]. Marketing claims for shea butter products emphasize their highly moisturizing properties due to its semi-solid characteristics, providing a buttery consistency and ease of spreading on the skin [19].

The result of spreadability test of Shea Butter samples and control (figure 2) showed quite similar pattern. Almost all samples were smooth and easy to be spread. One sample had small particles after spreading, this might be explained due to lack of good filtration or even rancidity of the sample. Spreading on hand might cause better result due to skin temperature.

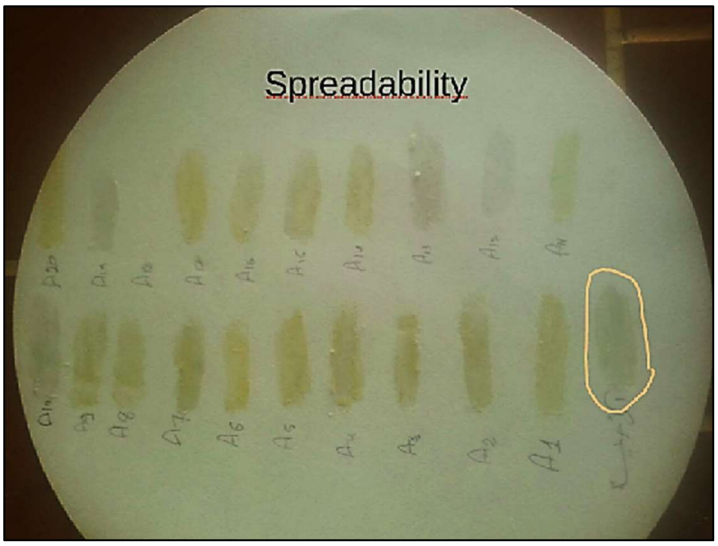

Figure 2: Spreadability test result of Shea butter control and samples

3.3 *Thin layer chromatography (TLC) chromatogram*

After dissolving Shea butter samples and control in hexane, they were spotted on TLC plate, then eluted in 8:2 hexane: ethylacetate twice to get good separation. After checking TLC plates under UV light, chromatogram showed very similar separation for all samples and control. The result indicates that the samples contained same ingredients and there is no foreign additives (figure 3).

The analysis of Shea Butter fatty acid methyl esters found triacylglycerides (TAGs) were comprised of four major fatty acids, palmitic, stearic, oleic, and linoleic acids, among which stearic and oleic acids were dominant ([20], [21]). This was similar to the resulted TLC chromatogram which suggest the presence of the same fatty acids in tested samples.

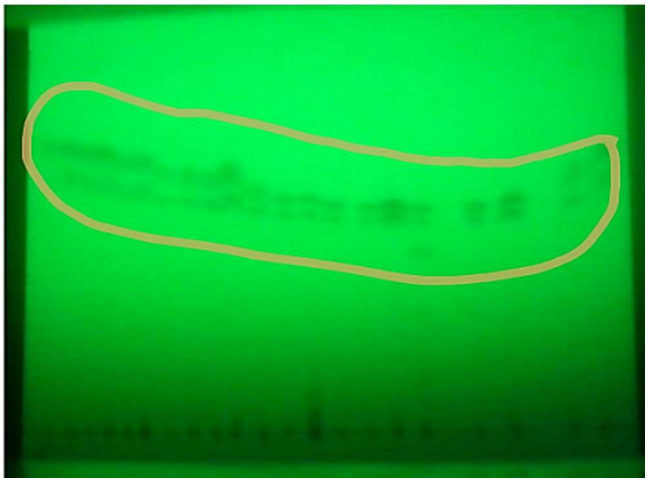

Figure 3: TLC chromatogram of Shea samples and control under UV eluted twice in 8:2 hexane: ethylacetate.

3.4 *Phytochemical screening test*

All performed tests to detect presence of reducing sugars, steroids and triterpenoids were positive tests. This result was compatible with previous result [13] which suggested the presence of the same ingredients.

3.5 *Antioxidant scavenging activity (DPPH) test*

It was noted that DPPH scavenging activities of control and samples compared to vitamin C were clear due to the change of color from violet to yellow (figure 4). This result indicates presence of antioxidant compounds in samples,

which would be due to the significant levels of vitamins A and E, that promotes strong antioxidant activity [22].

Shea Butter from Poland had intense yellow color that might be due to presence of some antioxidant additives such as grape seed oil, ascorbic acid, citric acid and coumarin. In addition to other fixed and volatile oils as listed in ingredients list. The result of DPPH scavenging activity in TLC chromatogram showed that samples contained more than one component responsible for antioxidant effect (figure 5).

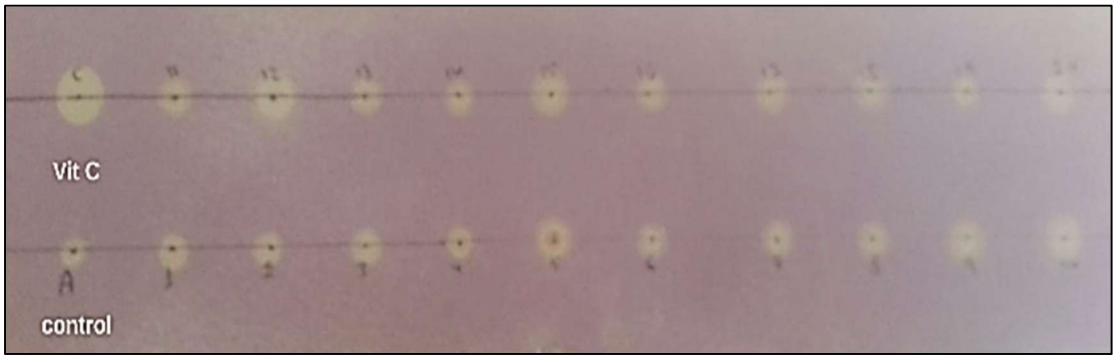

Figure 4: TLC spotted with Shea butter control and samples and sprayed with 0.2 % DPPH methanolic solution compared to Vit C (+ve control)

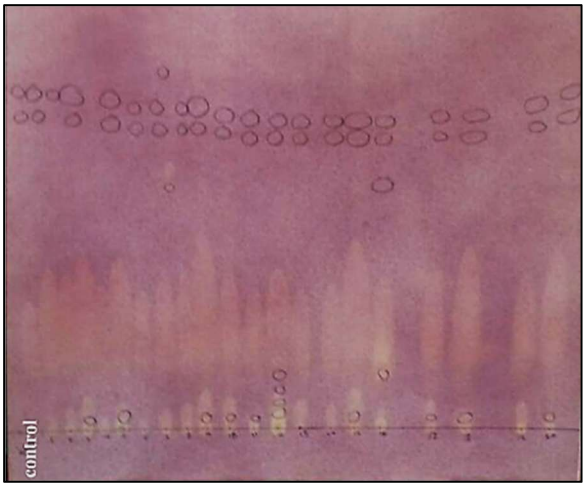

Figure 5: TLC plate of Shea samples and control eluted twice in 8:2 hexane: ethylacetate and sprayed with 0.2% DPPH methanolic solution

3.6 *Antimicrobial screening test*

Shea butter samples and control showed no antimicrobial effect against *Staph. aureus* and *P. aeruginosa* bacteria on Mueller-Hinton agar compared to ciprofloxacin used as positive control as shown in (figures 6 ,7). The tested organisms (pathogen) are based on their implication in human diseases such as skin diseases.

This result was compatible with a study [23] showed that *Pseudomonas aeruginosa* had resistance to both aqueous and ethanol shea seeds extracts. This was also supported by the antibacterial activities results of Shea seed extract and Shea seed oil against previously untested human pathogens *Staphylococcus aureus*, *Bacillus subtilis*, and *Listeria monocytogenes*, as well as an untested strain of *Escherichia coli*, were assessed using agar disc-diffusion and agar well diffusion assays. There was no antimicrobial activity against any of the tested bacteria [24]. On the other hand, the result of the antibacterial assay shown in [25] indicates that Shea oil seed extracted by hexane did not show any activity on *Pseudomonas aeroginosa*, *Escherichia coli* and *Trychophyton rubrum* but it shows slight activities on *Stapylococcus auerus*. The Petroleum ether extract showed reasonable activity on *Stapylococcus auerus* but inactive on *Pseudomonas aeroginosa*, *Escherichia coli*.

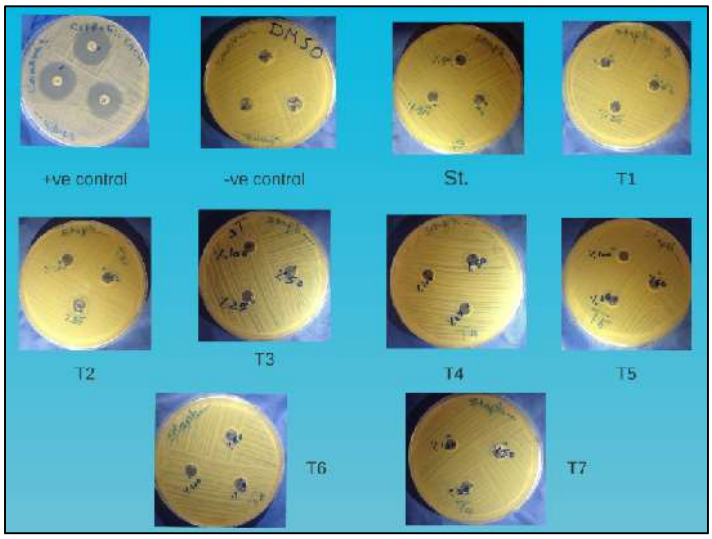

Figure 6: Antimicrobial screening test of controls and samples on Staph. aureus.

+ve control: Ciprofloxacin, -ve control: DMSO, St: control sample, T1-T7: random samples.

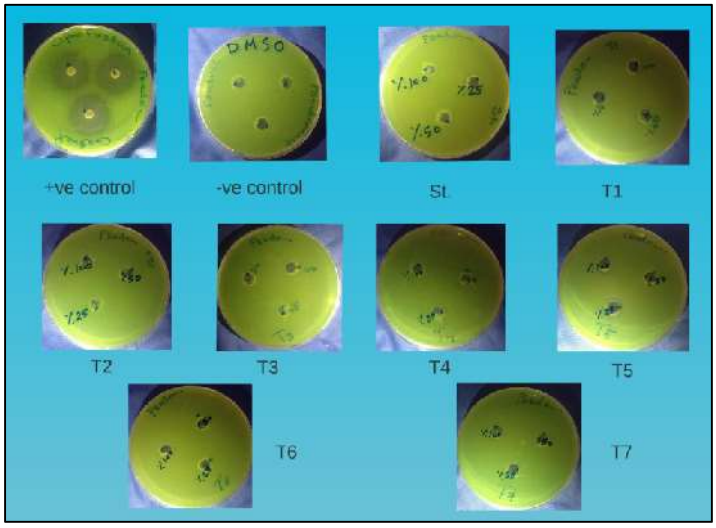

Figure 7: Antimicrobial screening test of controls and samples on P. aeruginosa.

+ve control: Ciprofloxacin, -ve control: DMSO, St: control sample, T1-T7: random samples.

IV. Conclusion

Shea butter samples showed wide diversity in colors and odors which is normal in unrefined raw Shea butter, while two samples were white in color suggesting exposure to refining procedures. Spreadability and TLC chromatograms were similar among samples and control. They also showed the presence of carbohydrates, steroids and triterpens. In addition to a good DPPH scavenging activity in qualitative level. Shea butter control and samples had no antimicrobial activities against *P. aeruginosa* and *S. aureus*. Thus, their antipathogenic activity shows that they cannot be exploited for use in pharmaceuticals.

Acknowledgment

The authors would like to thank University of Tripoli for providing facilities for this research.

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