African Journal of Biotechnology Vol. 10(65), pp. 14573-14582, 24 October, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1873
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Cosmeceutical values, antimicrobial activities and antioxidant properties of cashew leaves extract

Siavash Hosseinpour Chermahini* and Fadzilah Adibah Abdul Majid

Department of Bioprocess Engineering, Faculty of Chemical Engineering, Universiti Teknologi Malaysia, 81310 Skudai, Johor, Malaysia.

Accepted 2 August, 2011

With the increasing concern of public health and consumers' awareness, non-chemical cosmetics are booming worldwide. Plants are important in formulating natural and non-chemical cosmetic products, thus making the plant materials highly potential to be developed into cosmetic formulating ingredients. However, colour interference is a very critical item in producing cosmetic. The plant extracts which are normally dark brown and greenish colour, might spoil the appearance of the products if they are added in cosmetic formulation. In order to solve this problem, an activated carbon granule tea bag system was assembled and batch experiments were conducted as a mechanism to eliminate the intense colour of plant extract without affecting activities. The system was tested with different concentration of cashew leaves extract (5, 10, 15, 20, 25 and 30%) in contact with (10, 15, 20 and 25 g) granular activated carbon (GAC) from 0 to 6 h. Ethanol and water extracts of cashew leaves were compared for the cosmeceutical properties, mainly the antioxidant and antimicrobial activities. This study therefore suggests that cashew leaf extract is suitable to be used with ethanol for cosmetic application.

Key words: Antimicrobial, antioxidant, ethanol extract, water extract, cashew leaves, cosmeceutical.

INTRODUCTION

Cosmetics are products used to protect and improve the appearance of the skin or deodorate the human body. Cosmetics include skin-care creams, lotions, powders, perfumes, and so on. Today, with the growth of knowledge, consumers are moving towards the use of cosmetics from natural resources, more effective and with no chemical ingredients. They are looking for products without any side effects and at the same time more effective in maintaining the health of the skin. For this reason, some consumers use cosmeceutical products with combination of cosmetic and pharmaceutical ingredients to reduce or delay the process of skin aging, as well as maintenance of healthy skin by addition of photochemicals such as antioxidants. Antioxidants are components that are needed to protect the cells from aging caused by unstable molecules known as free radicals. Free radicals are highly reactive molecules or chemical species containing unpaired electrons that cause oxidative stress, which is defined as "an imbalance between oxidants and antioxidants in favor of the oxidants, potentially leading to damage" (Sies, 1997). Oxidative stress can damage lipids, proteins, enzymes, carbohydrates and DNA in cells and tissues, resulting in membrane damage, fragmentation or random cross linking of molecules like DNA, enzymes and structural proteins and even lead to cell death induced by DNA fragmentation and lipid peroxidation (Beckman and Ames, 1998).

These compounds are also claimed able to whiten (Sánchez-Ferrer et al., 1995) and rejuvenate the skin (Wolf et al., 1998; Pelle et al., 1999) mainly because of vitamin E, beta carotene, and vitamin C. The sources of the natural antioxidants are mostly from natural resources like plants, that are rich in phenolic compounds. As a matter of fact, plant extracts that are rich in antioxidants components will have high potential to be developed into cosmetic formulation. Natural ingredients such as *Anacardium occidentale* (Cashew) leaves extract could be an attractive candidate in cosmetic formulation due to these two important criteria: (a) antimicrobial activities

*Corresponding author. E-mail: siavash_hosseinpoor@yahoo.com. Tel: +6017866175 or +6075536778. Fax: +6075536778.

could minimise or replace the use of synthetic perservatives in cosmetics and (b) antioxidant properties could enhance skin nutrition as well as whiten the skin (Mary and Lupo, 2001). One of the challenges of using the plant parts in cosmetics formulation is the dark green colour of their extracts which will spoil the appearance of the final cosmetic products, besides its original odor.

Decolorisation is one of the approaches that can be applied to removed unwanted colours in the plant extracts. There are many methods available for decolorsation process. However, activated carbon is the most commonly used method of dye removal by adsorption (Nassar and El-geundi, 1991). This study focused on addressing decolorisation issues related to herbal extracts for cosmetic application.

Literature review

Cosmetics are commercially available products that are used to improve the appearance of the skin (Mary and Lupo, 2001). They have to provide functional and safe products (Kostarelos and Rheins, 2002). The last few decades have witnessed a great demand for herbal cosmetic products, away from synthetics. This is so because these herbal and natural cosmetics are safe to use and do not have any side effects. With man relearning the benefits of natural products, cosmetic is increasingly being used by leading herbal manufacturers, right from body lotions to face packs, from skin cleansers to toning creams. There has been a metamorphosis in the cosmetic industry with natural products being more in demand than their synthetic counterparts; this has been possible because of the shift in consumer preference from synthetic cosmetics to natural ones (Neem Associations, 2010).

Plant extracts and the use of plant parts such as leaves, fruits, flowers, stems, barks, buds and roots are known in cosmetic and pharmaceutical applications since ancient times. Applications of plants and plant extracts in cosmetics are wide spread and where used for purposes such as moisturizing, whitening, tanning, colour cosmetics, sunscreen, radical-scavenging, antioxidant, immunostimulant, washings, preservatives, thickeners etc. (Blum et al., 2007). It is thought that additional, topical use of vitamins (A, B, C, E and K) and antioxidants in cosmetics can better protect and possibly correct the damage by neutralizing these free radicals. In addition, some vitamins may be beneficial to the skin because of other actions such as effects of suppression of pigmentation and bruising, stimulation of collagen production, refinement of keratinization, or anti-inflammatory effects. Plants and plant derived ingredients are common and of major importance in the fields of pharmacy, food and cosmetics. The cosmetic industry is a fast moving market. Products have short lifecycles and the industry has to come up with innovative products constantly (Mary and Lupo, 2001). Plants are rich in a wide variety of secondary metabolites, such as tannins, terpenoids, alkaloids, and flavonoids, which have been found *in vitro* to have antimicrobial properties.

Antimicrobial ingredients are materials that protect against the growth of microorganisms in cosmetics and skin, including bacteria, viruses and fungi. Antimicrobial ingredients can also kill organisms that may be present in ingredients that may be used to make cosmetics. Antimicrobial ingredients play an important role in making sure that cosmetics are free of microorganisms during storage and after they are opened. They are effective at low levels so that it doesn't take much of the ingredient to work. There are many different types of materials that can be used in products and they are selected based on the specific type of product. A water-based product may use a different type of ingredient (or combination of ingredients) than an oil-based product (Council, 1994).

Clinical microbiologists have two reasons to be interested in the topic of antimicrobial plant extracts. First, it is very likely that these phytochemicals will find their way into the arsenal of antimicrobial drugs prescribed by physicians; several are already being tested in humans. It is reported that on average, two or three antibiotics derived from microorganisms are launched each year. Second, the public is becoming increasingly aware of problems with the overprescription and misuse of traditional antibiotics (Clark, 1996).

The cashew leaves and bark extract have great potential as an effective antimicrobial agent (Gaffar et al., 2008). Cashew's antimicrobial properties were first documented in 1982 *in vitro* study (Kudi et al., 1999). In 1999, researchers reported that cashew fruit exhibited antibacterial activity against the Gram-negative bacterium *Helicobacter pylori*, which causes acute gastritis and stomach ulcers (Kubo et al., 1999). Its effectiveness against leishmanial ulcers also was documented in two clinical studies (Franca, 1993; Frances et al., 1996). A study conducted by Akinpelu (2001) indicated that a bark extract exhibited *in vitro* antimicrobial activity against 13 to 15 microorganisms tested. The bark and leaves of cashew are a rich source of tannins, a group of plant chemicals with documented biological activity. These tannins, in a 1985 rat study, demonstrated anti-inflammatory (Mota, 1985).

Tannins have received considerable attention in the fields of nutrition, health and medicine, largely due to their physiological activity, such as antioxidant activity (Zhang and Lin, 2008), anti-microbial effects (Lim and Murtijaya, 2007), and anti-inflammatory properties (Sisti et al., 2008). Tannins as antioxidants often characterized by reducing power (Santos-Buelga and Scalbert, 2000) and free radical scavenging activities (Gulcin et al., 2003).

The extraction processing of the leaves fetch in the green chlorophyll. This dark green coloured extract usually contributed in unwanted greenish colour of end

products. In the processing perspective, pretreatment could be useful to eliminate green chlorophyll colour of the leaves (Gaffar et al., 2008).

MATERIALS AND METHODS

In the present method, the amount of cashew leaves powder to be used per 1 L ethanol or water, were 50, 100, 150, 200, 250, 300, 400, and 500 g. The 400 and 500 g are saturated and were not involved in this experiment, but are considered as limitation point. The colour intensity, antioxidant value, and antimicrobial value, were measured for every samples. Cashew leaves extract tested varying from 5 to 30% with intend of 5%. The amount of granular activated carbon (GAC) was 10, 15, 20 and 25 g. The decolorising process time was varied from 0 to 6 h. Two main data were collected for water and ethanol extract of cashew leaves (CL).

In all of the extracts (water and ethanol), 2 samples in 1.5 ml microcentrifuge tubes (from BIOLOGIX company) were taken every hour. Also, at the same time photos from all of samples were captured. After sampling, all samples were filtered 2 times with filter paper Whatman No.3, and then centrifuged at 8000 rpm for 5 min in order to separate AC from the extract.

Antioxidant

Folin's reagent is usually used to quantify the total phenolic and polyphenolic compounds present in plants, food, and beverages. The most easy, fast and simple analytical method is by using free radicals. A rapid and inexpensive example of these analytical methods is 2, 2-diphenyl-1-picrylhydrazyl (DPPH) method. It is now widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors and evaluate antioxidant activity of foods. It has been proved that it is an accurate, convenient and rapid way to determine antioxidant activity of various foods (Soto et al., 2008).

The reaction mixture contains 500 µl of test sample extracts and 500 µl of DPPH in ethanol. The concentration of DPPH is 0.0027 g in 50 ml ethanol in the reaction mixture. These reaction mixtures were kept in the dark for 30 min and the absorbance was measured at 570 nm (Liana-pathrianan and Shahidi, 2005).

Antimicrobial

To perform disc diffusion antimicrobial susceptibility testing, there are five major steps. These are sterilization, medium agar preparation, nutrient broth preparation, McFarland turbidity standard preparation and inoculums preparation. Muller-Hinton agar medium is the only susceptibility test medium that has been validated by NCCLS.

A suspension of the bacterial strain is adjusted to a standard density, and the suspension is swabbed evenly on a Muller-Hinton agar plate. Antimicrobial discs were applied to the inoculated surface of the Muller-Hinton plate. When bacterial multiplication proceeds more rapidly than the drug can diffuse, the bacterial cells that are not inhibited by the antimicrobial will continue to multiply until a lawn of growth is visible and no zone of inhibition appears around the disc. When the antimicrobial is present in inhibitory concentrations, no growth will appears in the zone around the disc. The more susceptible the bacterial strain tested, the larger the zone of inhibition.

The selection of microorganisms used in this study is based on the most commonly microorganisms found in cosmetics. The microorganisms tested in this study were *Staphylococcus aureus* (Gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria). *S. aureus* was used due to its clinical relevance as a major cause of hospital acquired infections of surgical wounds, and it rapidly develops resistance to many antimicrobial agents. These strains were prepared from CEPP laboratory. It was frozen at -80°C and was removed from the freezer 24 h before use. They were adjusted to approximatively 10^5 to 10^6 cells ml^{-1} using distilled water.

The growth method was performed as follows:

1. At least three to five well isolated colonies of the same morphological type were selected from an agar plate culture. The top of each colony was touched with a loop and the growth was transferred into a tube containing 4 to 5 ml of a suitable broth medium (tryptic soy broth for bacteria and 5 ml of distilled sterile water containing 0.1% Tween 20 for fungi and yeast).
2. The broth culture was incubated at 35°C until it achieved or exceeded the turbidity of the 0.5 McFarland standards (usually 2 to 6 h).
3. The turbidity of the actively growing broth culture was adjusted with sterile saline or broth to obtain turbidity optically comparable to that of the 0.5 McFarland standards. To perform this step properly, either a photometric device can be used or if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standards against a card with a white background and contrasting black lines.
4. Optimally, within 15 min after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.
5. The dried surface of an agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.
6. The lid may be left agar for 3 to 5 min, but no more than 15 min, to allow for any excess surface moisture to be absorbed before applying the drug impregnated discs.
7. The lid was then closed and labeled before being applied with the discs.

In the paper disc diffusion test, sterile paper discs were soaked in the different pre-treatment of cashew extracts for 2 h. 0.2 ml of a 24 h broth culture of each of the bacteria species were spread on the surface of gelled sterile Muller-Hinton agar plates. The paper discs containing different pre-treatment extracts were placed at different areas on the surface of each plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. The plates are inverted and placed in an incubator set to 37°C for 24 h within 15 min after the discs are applied. Antimicrobial inhibitory effects and activity of each extract against the test bacteria was indicated by growth-free "zone of inhibition" near the respective discs. Methanol and antibiotic were used as negative and positive control, respectively.

The procedure for the experiment is indicated below:

1. The plates were inverted and on the outside of the plate, the diameter of the zone of inhibition were measured in centimeters (including the disc). The lid of the Petri dish should not be removed.
2. The results were recorded in the laboratory.
3. The measurements were compared to the zone-size interpretive chart to determine whether the organism was resistant,

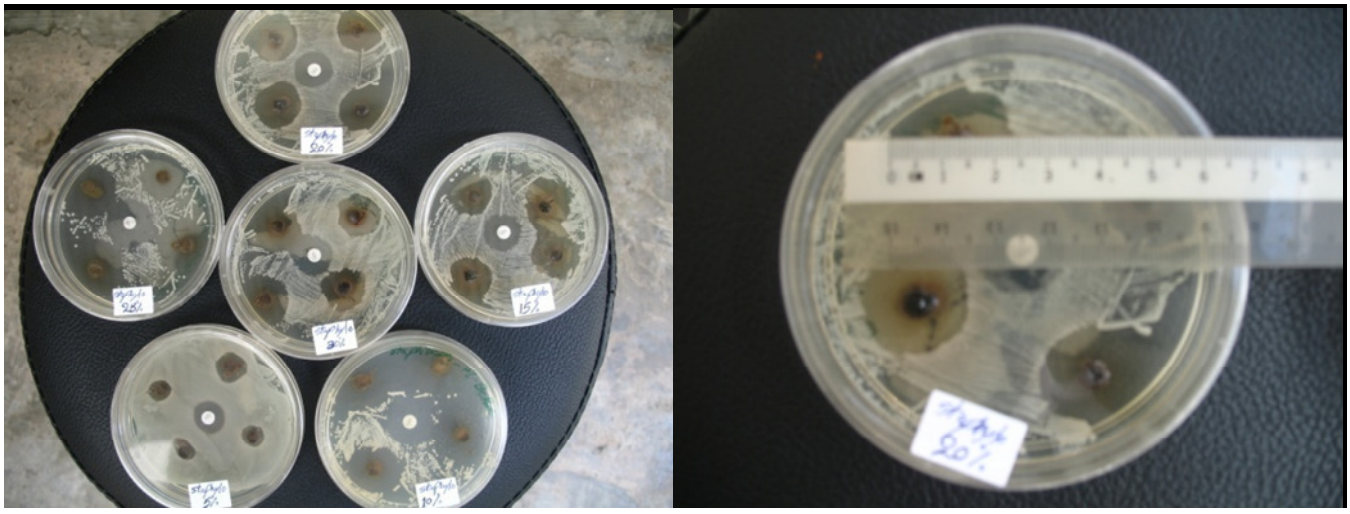

Figure 1. Inhibition zone of different concentration of cashew leaves.

intermediate or susceptible to the antibiotics.
4. Activity index was calculated by comparing the zone of inhibition of sample with standard antibiotics, using the formular:

$$\text{Activity index} = \frac{\text{Inhibition area of test sample}}{\text{Inhibition area of standard antibiotics}}$$

RESULTS AND DISCUSSION

Different concentration of cashew leaves extract (5, 10, 15, 20, 25 and 30%) (Figure 1), in water and ethanol were treated by different amount of GAC. Duration of treatment was 6 h and sampling time was for every 1 h. The amount of GAC was 10, 15, 20 and 25 g. Mode of measurement was OD 340 nm (primary wavelength) for water and 630 nm for ethanol. Observation was made based on changes in the colour with time intensity.

A. occidentale (cashew) leaves is rich in phenolic compounds and contain various flavonoid (Raghavan, 2007). Previous studies proved that the extract of cashew leaves exhibited particularly strong antioxidant activities and showed strong activity in the free radical scavenging assay (Kadirvelu and Namasivayam, 2003). These characteristics make it very potential to be developed into an ingredient for cosmeceutical formulation. However, the challenge is the dark brown and greenish colour of the plant extracts. Colour of plant extracts make it not suitable to be used as an ingredient of cosmeceutical products since it affects the appearance of the products. In order to solve this problem, batch decolorisation treatment was carried out on the samples by using different volume of GAC with different contact time.

From the Figures 2 and 3, we can observe the effect of decolorisation by using activated carbon. The colour reduction, which was based on the values of OD (Optical Density), gradually increased with time. The larger the difference between initial and final value of OD, the more effective the colour of sample reduced (that is higher adsorption efficiency). Mode of measurement was OD 340 nm (primary wavelength) for water and 630 nm for ethanol.

Antioxidant assay for DPPH free radical scavenging activity (RSC)

As aforementioned, the concern of this study is not only to reduce the green colour of cashew leaves but also to maintain the quality of extract. High amount of antioxidant is the main reason for this purpose because the cashew leaves contain high potential antioxidant to be develop into cosmeceutical formulation ingredient. Although, most antioxidant activities from plant sources are derived from phenolic-type compounds, this does not indicate that the presence of small quantities of phenolics will result in low antioxidant activities. Therefore, in this section, DPPH free radical scavenging activity was carried out to investigate if the antioxidant property of extracts was being maintained after the treatment which reduced the colour and quantities of phenolics in the samples. DPPH is one of the commonly used substrate for fast evaluation of antioxidant activity because of its stability (in radical form) and simplicity of the assay. In this assay, the ability of investigated sample extracts to act as donors of hydrogen atoms or electrons in transformation of DPPH radical into its reduced form DPPH-H was investigated (Babji et al., 2005). If the sample is able to turn the stable purple coloured radical solution into yellow colour solution, then it has the ability to scavenge free radical.

Figure 4a shows that for 5% cashew leaf extract (CLE) reduction in antioxidant value starts after 1 h mostly for 15 and 20 g GAC. However, it is constant until end of experiment (6 h). Figure 4b indicates that there is no

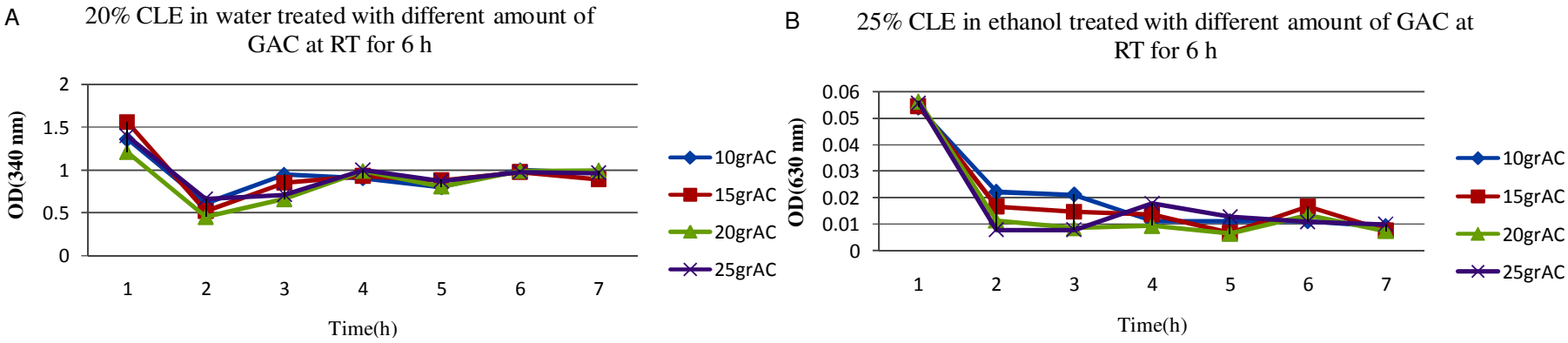

Figure 2. Graph of OD 340 nm for water and 630 nm for ethanol versus contact time.

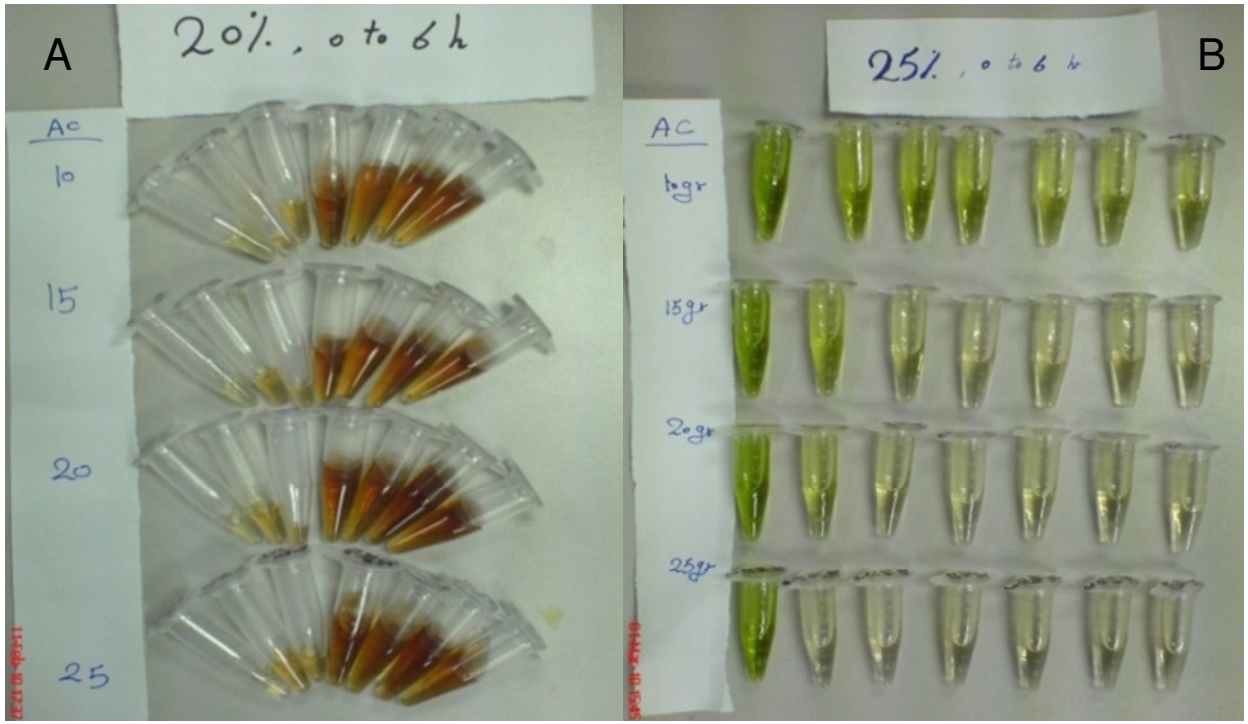

Figure 3. Colour changes for water before 2 h (A) and for ethanol after 1 h (B).

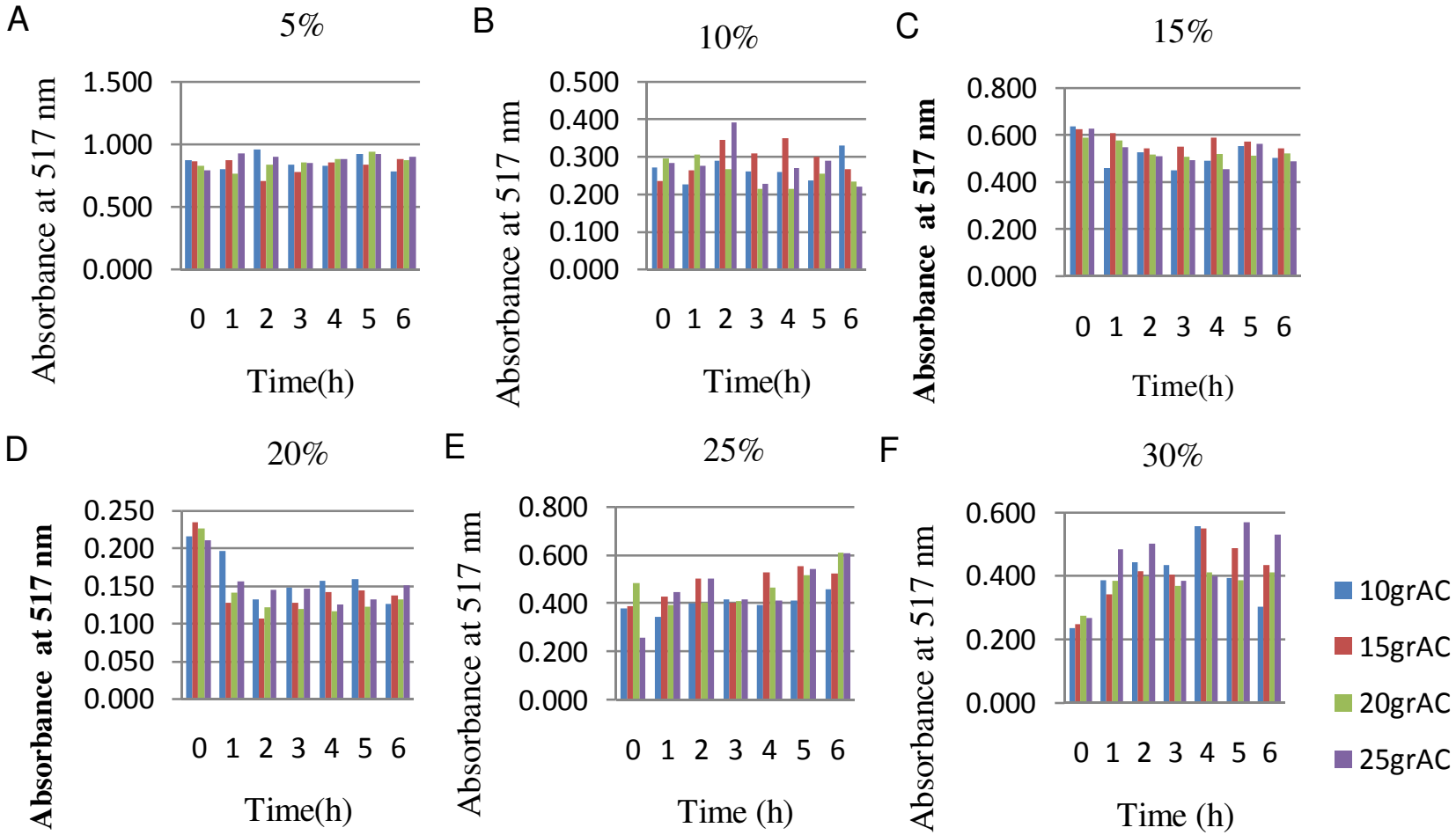

Figure 4. Antioxidant activity at different contact time for treatment of different concentration CLE in water with different amount of GAC.

significant reduction in antioxidant value after treatment of 10% CLE. Figure 4c presents that antioxidant value decrease until 2 h treatment of 15% CLE, then remained constant. In Figure 4d, there is a significant reduction in antioxidant value within first hour treatment of 20% CLE (water) then remain constant until end of experiment (6 h). Figure 4e shows that significant reduction in antioxidant occured after 1 h of treatment, but exposing extract to GAC for more than 5 h gave false reading (increase in absorbance value) which is illogical. Figure 4f shows that after 1 h of treatment, antioxidant level seems to increase. The increase in absorbance could be due to the release of powdered GAC into the solvent. Since

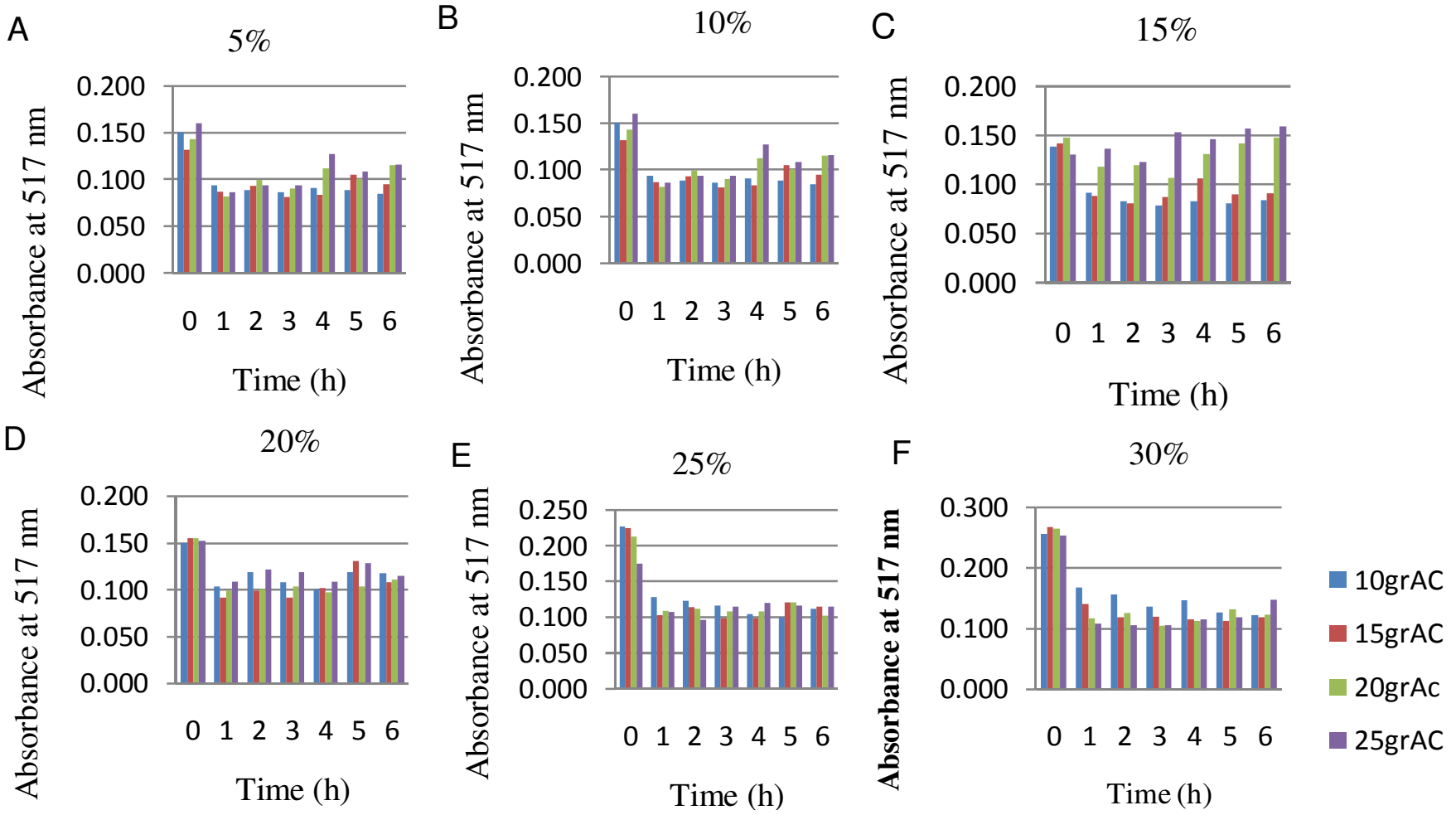

Figure 5. Antioxidant activity at different contact time for treatment of different concentration CLE in ethanol with different amount of GAC.

the CLE concentration is high (30%) and did not produce good colour reduction, this concentration was not selected for further study.

In Figure 5a, there is a significant reduction in antioxidant value after the first hour of treatment of 5% CLE, afterwhich the antioxidant value remained constant. Figure 5b shows that there is a significant decrease in antioxidant value within first hour treatment of 10% CLE. Antioxidant value after that remain constant. In Figure 5c, there is a significant reduction in antioxidant value mostly for 10 and 15 g GAC, which remained constant until end. For 25 g GAC there is no decrease. Figure 5d shows that there is a significant reduction in antioxidant value within first hour and then remain almost constant until end of experiment. Figure 5e indicates a sharp decrease in antioxidant value from the first hour of treatment of 25% CLE.

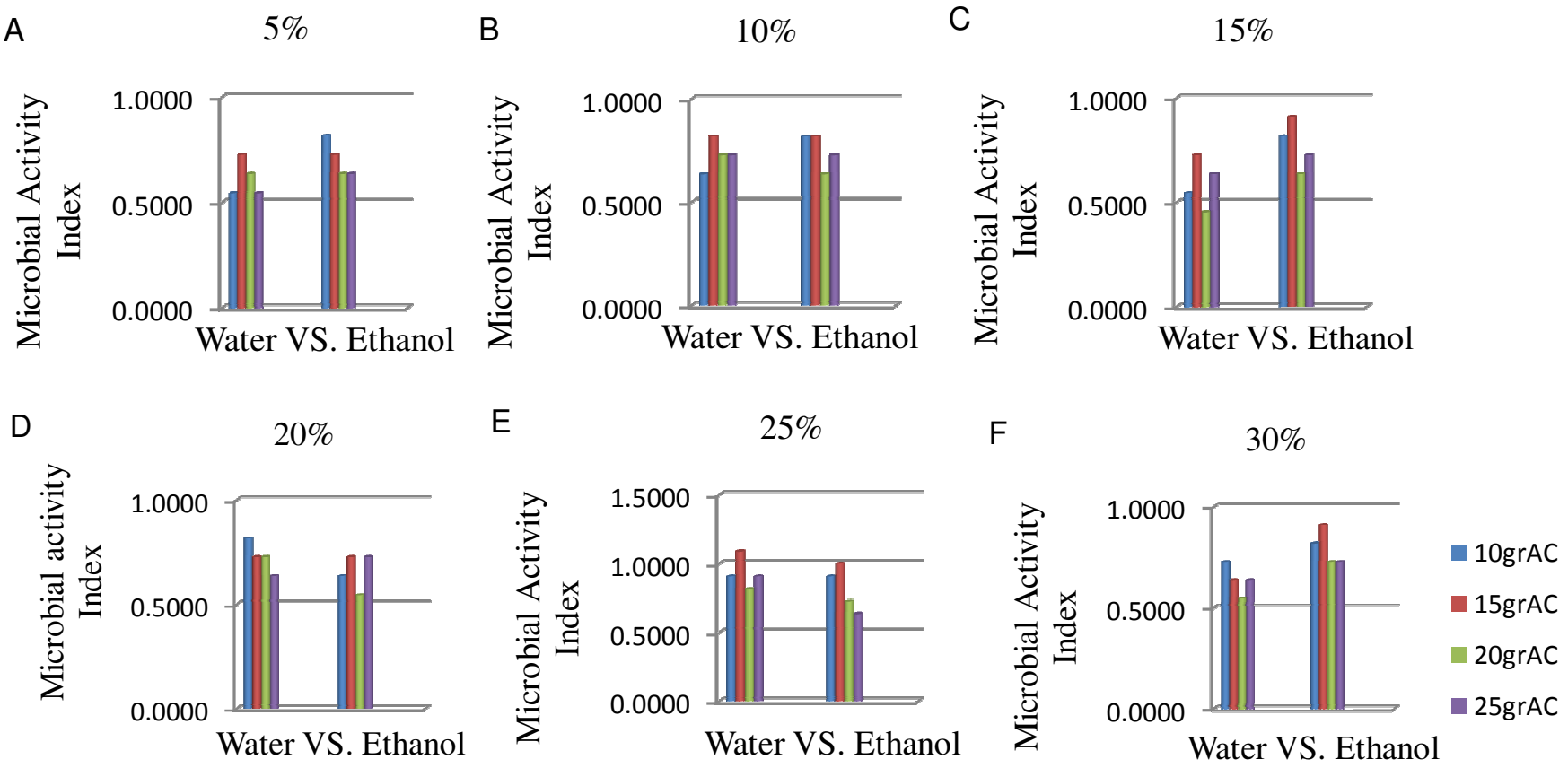

Figure 6. Microbial activity index of different concentration of CLE in water or ethanol.

Antioxidant value remain constant after 1 h until the end of experiment. Figure 5f presents that there is a significant reduction in antioxidant value within the first hour and then until end of experiment, antioxidant value is constant.

Antimicrobial activities

Antimicrobial test was carried out to select the suitable concentration of CLE and the suitable amount of activated carbon for decolorisation process. The microorganisms were *E. coli* for Gram-negative and *S. aureus* as Gram-positive. According to observations (experiment repeated 3 times); no clear zone for *E. coli* was observed. This means that antimicrobial activities of cashew leaves did not have any effect on Gram-negative bacteria. The experiment continued with the testing on Gram-positive bacteria which is *S. aureus.* Microbial activity index (MAI) was also calculated.

Figure 6a shows that antimicrobial activity of 5% CLE in ethanol is higher than water. Figure 6b indicates that antimicrobial activity in both water and ethanol are almost equal, especially for 10 and 25 g GAC. In Figure 6c, antimicrobial activity for 15% CLE for ethanol is significantly higher than for water. Figure 6d presents that antimicrobial activity in 20% CLE for water is more than for ethanol. In Figure 6e, with increasing a mount

of GACfor ethanol, antimicrobial activity decreased. But microbial activity index for both water and ethanol is around 1.00 mm or more. Finally, in Figure 6f, antimicrobial activity in 30% CLE for ethanol is significantly higher than for water. This is obvious in 15 g GAC.

Economical discussion

Water is cheap and could be used to produce extract for oral consumption. However, there is a limitation for the use of water because it is not a good preservative. On the other hand, ethanol is more expensive, but good preservative. Ethanol extract may have wider applications but it has *halal* issue for certain applications. After all the experiments (antimicrobial tests and antioxidant properties), we can conclude that each solvent has its unique properties. For instance, 25% concentration is suitable for ethanol and 20% is suitable for water.

Conclusion

This research gives an account of and the reasons for the widespread use of cashew leaves extraction in cosmetic. The purpose was to improve the appearance of cosmetic product that contains natural plant extracts as ingredients, in order to increase the acceptance of consumers. The evidence from this research suggests that 15 g of granular activated carbon was chosen as the best amount of activated carbon to be used as tea bag to treat 20% of cashew leaves extract. The suitable operation contact time for ethanol is after 1 h, and for water before 2 h. Under the stated conditions, the designed tea bag was able to function efficiently for all concentration of cashew leaves with different amount of GACs. The treatment carried out by using the designed tea bag was able to improve the colour and appearance of extract with minimal affects on antioxidant properties and antimicrobial activities.

In conclusion, the concentration of CLE, amount of GAC, and also the best contact time for treatment with GAC based on all of the experiments and calculations to improve the colour and appearance of extracts with minimal affects on antioxidant properties and antimicrobial activities of the extracts are: 20% for water and 25% for ethanol (depending on the application), 15 g GAC (which is the best choice for decolorisation), and 1 h, respectively.

REFERENCES

Abdul Gaffar R, Abdul Majid FA, Sarmidi MR (2008). Tyrisonase Inhibition and Melanin Reduction of Human Melanocytes (HEMn-MP) using *Anacardium occidental* L Extract. Med. J. Malay., p. 63

Abdul Gaffar R, Sazali NES, Abdul Majid FA (2008). Colour Reduction and Anti-Microbial Evaluation of Pre-Treated Cashew Leaves Extract. J; Chem. Nat. Resour. Eng., 2: 1-9.

Akinpelu DA (2001). Antimicrobial Activity of *Anacardium Occidentale* Bark. Fitoterapia, 72(3): 286-87.

Babji, A. S., Noriham, A. and Chan, K. W. (2005). Phytogenic Antioxidants in Processed Chicken Products. PPM, 1: 26-28.

Beckman KB, Ames BN (1998). The Free Radical Theory of Aging Matures. Physiol. Rev., 78: 547-581.

Blum P, Schurch C, Zulli F (2007). Topische Anwendung von dedifferenzierten Pflanzenzellen furden Schutz und die Erneuerung von Hautstammzellen. Patent pending.

Clark AM (1996). Natural Products As A Resource For New Drugs. Pharm. Res. 13: 1133-1144.

Council (1994). Safety of cosmetic and personal care products. The council is the trade association representing the cosmetic, toiletry and fragrance industry: Washington, FROM http://www.cosmeticsinfo.org/php.

Franca F (1993). An Evaluation of the Effect of A Bark Extract from the Cashew (*Anacardium Occidentale* L.) on Infection by Leishmania (Viannia) Braziliensis. Rev. Soc. Bras. Med. Trop., 26(3): 151-55.

Frances F, Lago EL, Marsden PD (1996). Plants Used in the Treatment of Leishmanial Ulcers Due to Leishmania (Viannia) Braziliensis in An Endemic Area of Bahia, Brazil. Rev. Soc. Bras. Med. Trop., 29(3): 229-32.

Gulcin I, Oktay M, Kirecci E, Kufrevioglu OI (2003). Screening of Antioxidant and Antimicrobial Activities of Anise (*Pimpinella anisum* L.) Seed Extracts. Food Chem., 83: 371-382.

Kadirvelu K, Namasivayam C (2003). Activated Carbon from Coconut Coirpith as Metal Adsorbent: Adsorption of Cd (II) from Aqueous Solution. Adv. Environ. Res., 7: 71-478.

Kostarelos K, Rheins L (2002). Biotechnology Impacting Cosmetic Science: Altering the Way Cosmetics Are Perceived. Cosmet. Toiletries, p. 34.

Kubo J, Lee JR, Kubo I (1999). Anti-Helicobacter Pylori Agents from the Cashew Apple. J. Agric. Food Chem. 47(2): 533-537.

Kudi AC, Umoh JU, Eduvie LO, Gefu J (1999). Screening of Some Nigerian Medicinal Plants for Antibacterial Activity. J. Ethnopharmacol. 67(2): 225-28.

Liana-pathrianan CM, Shahidi F (2005). Antioxidant Activity of Commercial Soft and Hard Wheat (*Triticum aestivum* L) as Affected By Gastric PH Conditions. J. Agric. Food Chem. 53: 2433-2440.

Lim YY, Murtijaya J (2007). Antioxidant Properties of *Phyllanthus Amarus* Extracts As Affected by Different Drying Methods. LWT-Food Sci. Tech., 40: 664-1669.

Mary P, Lupo MD (2001). Antioxidants and Vitamins in Cosmetics. Clin. Dermatol., 19: 467–473.

Mota ML (1985). Anti-Inflammatory Actions of Tannins Isolated from the Bark of *Anacardium occidentale* L. J. Ethnopharmacol., 13 (3): 289-300.

Nassar MM, El-Geundi MS (1991). Comparative cost of colour removal from textile effluents using natural adsorbents. J. Chem. Technol. Biotechnol., 50: 257–264.

Neem Associations (2010). Neem Leaf, JUN, 19, 2010, from http://www.neem-products.com/neem-leaves.html.

Pelle E, Muizzudin N, Mammone T (1999). Photoprotection against endogenous and UVB-induced oxidative damage in stratum corneum lipids by an antioxidant-containing cosmetic formulation. Photodermatol. Photoimmunol. Photomed., 15: 115-119.

Raghava S (2007). Handbook of Spices, Seasonings and Flavourings, Second Edition, United States of America: CRC Press, Taylor and Francis Group.

Sánchez-Ferrer A, Rodríguez-López JN, García-Gánovas F, García-Carmona F (1995). Tyrosinase: A Comprehensive Review of Its Mechanism. Biochim. Biophys. Acta 1247: 1–11.

Santos-Buelga C, Scalbert A (2000). Proanthocyanidins and Tannin-Like Compounds-Nature, Occurrence, Dietary Intake, and Effects on Nutrition and Health. J. Sci. Food Agric. 80: 1094-1117.

Sies H (1997). Oxidative Stress: Oxidants and Antioxidants. Exp. Physiol., 82: 291-295.

Sisti M, De Santi M, Fraternale D, NinfalinP, Scoccianti V, Brandi G (2008). Antifungal Activity of Rubus Ulmifolius Schott Standardized *in vitro* Culture. LWT-Food Sci. Tech., 41: 946-950.

Soto ML, Moure A, Domínguez H, Parajó JC (2008). Charcoal

Adsorption of Phenolic Compounds Present in Distilled Grape Pomace. J. Food Eng., 84: 156-163.
Wolf R, Wolt D, Ruocco V (1998). Vitamin E: the radical protector. J. Eur. Acad. Dermatol. Venereol., 10: 103-117.
Zhang LL, Lin YM (2008). HPLC, NMR and MALDI-TOF MS Analysis of Condensed Tannins from Lithocarpus Glaber Leaves with Potent Free Radical Scavenging Activity. Molecules, 13(12): 2986-2997.