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Design and evaluation of cream-based formulations containing two organic materials for adsorbent, antimicrobial, antioxidant and anti-inflammatory properties

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Activated charcoal and turmeric (*Curcuma longa* L.) powders, commonly used as medicines or spices, are considered valuable anti-aging agents. The aim of the study is to formulate activated charcoal and turmeric powders into Aqueous cream BP and assess for adsorbent, antioxidant, anti-microbial and anti-inflammatory properties. Activated charcoal and turmeric were characterized and twenty creams prepared with each powder alone and combined, were evaluated for organoleptic properties, viscosity, spreadability, antioxidant activity, stability and antimicrobial properties. Anti-inflammatory activity was evaluated using carrageenan-induced acute inflammatory model. Data was analyzed using ANOVA at $\alpha_{0.05}$. Creams containing 6%w/w activated charcoal and turmeric powders 3:1 gave comparable ($p = 0.001$) antioxidant and anti-inflammatory properties with the marketed sample and promising antimicrobial activities against Gram-positive and Gram-negative bacteria, and fungi. No change in stability occurred throughout the period of storage. Combination of turmeric and activated charcoal powders showed antioxidant, anti-microbial, anti-inflammatory, and adsorbent properties in cream formulations.

Keywords: Activated charcoal powder, Adsorbent, Anti-inflammatory property, Anti-microbial, Antioxidant, Aqueous cream BP, Turmeric powder

INTRODUCTION

The major cause of skin aging is chronic exposure to UV rays, inducing harmful effects on skin and prompting oxidative damage to DNA, lipids, and proteins via the overproduction of free radicals[1]. Characteristic signs of skin aging include the loss of skin elasticity and wrinkling due to decreased levels of collagen production and rapid collagen breakdown[2,3]. Antioxidants neutralize free radicals, unstable oxygen molecules that break down skin cells and cause wrinkles, thus preventing impairment at the cellular level. Besides endogenous antioxidants present in human skin (e.g., glutathione, superoxide dismutase, catalase), exogenous antioxidants, normally administered using topical formulations, may also exert a key role in mitigating the biochemical consequences of oxidative stress by preventing protein and lipid oxidation, enhancing DNA repair, and scavenging free radicals[4].

The functions of skin creams are to protect the skin against harshness from the environment, restore moisture, allow the elimination of waste matter through the pores, and cool the body by evaporation of water (perspiration) and radiation, thus aiding in the maintenance of the normal body temperature. A skin cream should aid the skin in carrying out its normal functions. Creams provide a barrier to protect the skin as well as having cleansing and emollient properties[5]. Combined exposure of toxic chemicals such as acrylamide from synthetic skin formulations and cosmetics adds to daily exposure to hazardous chemicals from air, water, food and other sources. This explains why the demand for new cosmetic formulations, mostly derived from botanicals and other organic sources, has grown; the main attraction being their non-toxicity and their ability to counteract skin aging signs[1,6]. These organic products are materials derived from natural sources and are thought to be devoid of harmful chemical ingredients, therefore having

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no significant deleterious effect on the skin. Two of such organic ingredients that may be found useful in skin care are activated charcoal and turmeric powders.

Organic activated charcoal is made by adding calcium chloride or lemon juice to carbon-rich materials such as wood, peat, coconut shells or sawdust and subjecting the mixture to a very high temperature. The activation process strips the charcoal of previously absorbed molecules and frees up bonding sites again. This process also reduces the size of the pores on the charcoal and makes holes in each molecule, therefore increasing its overall surface area. Activated charcoal is a good adsorbent with the ability to adsorb dirt and toxin from surfaces. Activated charcoal provides anti-bacterial, anti-fungal properties; deep pore cleansing, oil-balancing and gentle exfoliating to skin, therefore giving the overall effect of a smoother and more refined appearance[7]. The most important feature of the activated charcoal-based skin care products is its desirable anti-aging (anti-inflammatory and antioxidant) and antimicrobial effects on the skin.

Thus, the aim of this study is to design cream formulations of Aqueous cream BP containing the two organic materials, activated charcoal and turmeric powder, alone and combined, and evaluate these formulations for adsorbent, antimicrobial, antioxidant, and anti-inflammatory properties**.**

MATERIALS AND METHODS

Materials

The materials used included emulsifying wax anionic (Niram Chemicals Ltd, India), Chlorocresol (BDH Limited Poole England), Methylene blue (BDH, limited Poole England). Sodium hydroxide (SD Fine-Chem Ltd, India), Sulphuric acid (Trident Ltd, India), Ferric chloride (Hosea Chem Industry, China), Wagner's reagent (Loba Chemie PVT Ltd, Mumbai, India) and Chloroform (Dow Chemical Group, USA). Activated charcoal powder (Kunimed Pharmachem Ltd, Ikeja, Nigeria), Carrageenan powder (Sigma-Aldrich, St Louis, MO, USA), Diclofenac powder and Gentamicin adsorption capacity, being able to remove large variety of particulates or contaminants, which can be either organic or inorganic[8.9]. Turmeric, *Curcuma longa* (Family Zingiberaceae), is a perennial herbaceous plant of the ginger family. Turmeric is an organic product with numerous health benefits reported and these include anti-bacteria, anti-fungal, anti-inflammatory, anti-aging, and antioxidant properties[10,11]. The health benefits of the rhizome are primarily due to the presence of curcumin, a bioactive component of the yellow pigments isolated from turmeric and is a major ingredient of the spice curry. Curcumin possesses many bioactivities, such as antioxidant, anti-inflammatory, antiviral, antifungal, cancer chemo-preventive and cancer chemotherapeutic properties[12-14]. Anti-inflammatory agents are known to fight inflammation while antioxidants counter the effects of free radicals. These properties of curcumin in turmeric suggest that the rhizome powder could be incorporated into a cream base for therapeutic purpose and can be explored to produce cream formulations with creams (Drugfield Pharmaceutical limited, Sango Otta, Nigeria) and Tioconazole cream (Nemeith International Pharmaceuticals Plc, Oregun Industrial Estate, Oregun Lagos, Nigeria). Turmeric rhizomes were obtained from farmers in Bodija Market, Oyo State, Nigeria.

Methods

Preparation of turmeric powder

Turmeric rhizomes were thoroughly washed with distilled water, peeled, washed again, and cut into small pieces. The pieces were air-dried for 10 days. The dried rhizome pieces were pulverized using a laboratory blender, screened through a mesh sieve of 125 µm and then stored in an air-tight container at room temperature (27±2^{o}C).

Characterization of activated charcoal and turmeric powders

Phytochemical analysis

Phytochemical analysis was carried out to screen for the presence of flavonoids, cardiac glycosides, tannins, saponins, alkaloids and anthraquinones using standard methods[15-20]

Particle size determination

Particle size of each powder was determined by analyzing 100 particles using an optical microscope. The average particle size was calculated using Edmundson's equation.

$$Dmean = \sum nd / \sum n \qquad \text{(Equation 1)}$$

where, n = number of particles analyzed; d = mean size.

Angle of repose

The angle of repose gives a qualitative assessment of the flowability of the powders. Powder sample was allowed to flow freely through a funnel under gravity into an open-ended cylinder placed on a base of similar diameter allowing a conical heap to be formed. The angle of repose was calculated from the formula:

$$Tan\ \theta = h/r \qquad \text{(Equation 2)}$$

where h is the height of the powder and r is the radius of the base of the cone. Determinations were dome in triplicate.

Swelling index

Swelling index was determined by transferring 10 g of powder into a 100-mL measuring cylinder and noting the volume occupied (V_1). Distilled water (90 mL) was added; the dispersion was shaken for 2 min and then made up to volume. The slurry was left to stand for 24 h before the sedimentation volume was read (V_2). The swelling index was then calculated using the formula:

$$\frac{V2 - V1}{V1}\ x\ 100 \qquad \text{(Equation 3)}$$

Determinations were made in triplicate.

pH determination

The pH of a 1.0 % w/v aqueous solution of powders was determined using the pH/meter (Model 720 A, Thermo Electron Corporation, MA, USA). Determinations were made in triplicate.

Densities

The particle density of the powders was determined using the pycnometer bottle method with xylene, a suitable non solvent as the displacement fluid. The bulk density of a given mass of powder (20.0g) was obtained by determining the initial volume occupied by the powder when allowed to pour freely through a funnel into a 100 mL graduated measuring cylinder. The tapped density was obtained following tapping of each powder in the 100-ml measuring cylinder at a standardized rate, 38 taps per min. Determinations were made in triplicate.

Adsorption properties of activated charcoal powder

The adsorption of methylene blue dye (MB) by activated charcoal powder was evaluated using amber-colored bottles covered with black paper to avoid reaction with sunlight. The adsorbent dose of 0.1 gm. of sample was placed in a beaker which contained 50 mL of a 50mg/L concentration of MB. The solution was then vibrated at 200 rpm, centrifuged at 700 rpm and decanted. The residual concentration of MB was measured by a UV–vis spectrophotometer at a pre-optimized wavelength (λ_{max}) of 665nm. The percent adsorption of MB was calculated by the following equation:

$$\%\ A = Ci - Cf\ /\ Ci\ x\ 100 \qquad \text{(Equation 4)}$$

Where *% A* is percentage adsorption while Ci and Cf are initial and final MB concentrations, respectively. Determinations were made in triplicate.

***In vitro* antimicrobial activities of turmeric and activated charcoal powders**

Preparation of powder samples

Sample powder (0.2g) was weighed and dissolved in 2.0 mL of dimethyl sulfoxide (DMSO) to prepare 10µg/ml. Gentamicin and Tioconazole were used as controls for bacteria and fungi, respectively. The test organisms used

were *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Klebsiellae pneumonia*, *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifera* and *Penicillin notatum*.

Determination of antibacterial activity (pour plate method)

An average culture of each organism was prepared by taking a loopful of the organism from stock and inoculating each into the sterile nutrient broths (5.0 mL each) followed by incubation for 18 - 24 h at 37^{0}C. From this overnight culture, 0.1 mL of each organism was obtained and transferred into 9.9mls of sterile distilled water to get a 1:100 dilution of the organism. From the diluted organism (1:100), 0.2 mL was taken into the prepared sterile nutrient agar (45^{0}C), then aseptically poured into sterile petri dishes, and allowed to solidify for about 45 - 60 min using a sterile cork-borer of 6 mm diameter. The wells were made in duplicate. The plates were left on the bench for 2 h to allow pre-diffusion and then incubated uprightly for 18 - 24 h at 37^{0}C. The bacteria plates were observed after 24 h for zones of inhibition.

Determination of antifungal activity (surface plate method)

Sterile Sabouraud Dextrose Agar (62g/L) was prepared and aseptically poured into the sterile plates in duplicate and allowed to set properly. After setting, 0.2 mL of the 1:100 dilution of organisms was transferred using sterile spreader to cover all the surface of the agar. Wells were made using a sterile cork-borer of 8mm diameter. In each well, graded concentrations of the samples were introduced including the controls. The plates were left on the bench for 120 min to allow for pre-diffusion. The fungi plates were then observed after 48 h of incubation.

Preparation of aqueous cream-based formulations containing activated charcoal and turmeric powders

Aqueous cream BP (100g) was prepared by dissolving 0.1g of chlorocresol in purified distilled water (69.9 mL) by heating on a water bath. Emulsifying ointment (30g) was weighed, transferred to a porcelain dish and melted on a water bath. The solution of chlorocresol, whilst still hot, was added to the melted ointment and stirred gently to form Aqueous cream BP. Turmeric powders alone, activated charcoal alone, and combinations of the two (at ratios 2:1 and 3:1) were incorporated into the cooled cream with continuous stirring for 15 min to produce twenty different cream formulations with concentrations 2.0, 3.0, 4.0, 5.0, and 6.0%w/w as presented in Table 1.

Characterization of cream formulations

Organoleptic properties

The creams were observed visually for color, perceived for odor, while 1mL of each sample was rubbed at the back of the palm to evaluate texture.

Antioxidant activity

In this study, 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was used to measure the scavenging ability of the cream formulations. The concentrations of formulations were fixed as 5000, 2500, 1250, 625, and 312 µg/mL. The positive control was L-ascorbic acid (0.1-50µg/mL diluted in water). Sample cream (2.0 g) was mixed with 2.0 mL of distilled water, vortexed, and sonicated for 30 min to obtain a clear solution. This was then mixed with DPPH solution and the change in optical density of DPPH radicals was monitored[21-22]. The sample mix (1.0 mL) was diluted with 1mL of DPPH solution (0.3mM). After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation below:

$$\%\, I = Abr - Aar/Abr \times 100 \quad \text{(Equation 5)}$$

Where i is the percentage inhibition, *Abr* is the absorbance of control and *Aar* is the absorbance of the sample reaction that had taken place. Determinations were made in triplicate.

In vitro antimicrobial activity of cream formulations

Cream sample (0.2g) was weighed and dissolved into 2.0mL of dimethyl sulfoxide to prepare 10µg/ml. Gentamicin and Tioconazole were used as controls for bacteria and fungi, respectively. The pour plate methods and surface plate methods earlier described were used for evaluating the antibacterial and antifungal activities of the formulated creams, respectively.

pH

The pH of cream formulations was determined using a pH meter (Model 720 A, (Thermo Electron Corporation, MA, USA) equipped with a glass electrode previously calibrated.

Viscosity

The viscosity of the formulations was determined at room temperature (25.0^{0}C) using a Brookfield viscometer with spindle size 7 (Brookfield Engineering Laboratories, Inc., Stoughton) at different rotational speeds.

Spreadability

Cream formulation was sandwiched between two solids, of dimension 20 ×5 cm, by placing a weight of 100 g on the upper solid. The weight was removed and any excess cream was scraped off. The solids were fixed to a stand at an angle of 45˚ angle such that only the lower slide was held firmly by the clamp, allowing the upper slide to slip off freely under a weight of 20g. The time taken for the upper solid to separate from the lower glass plate was noted. Experiments were done in triplicate and spreadability was calculated as shown below:

$$S = WL\,X\,L/T \qquad \text{(Equation 6)}$$

where S = spreadability; W= weight tied to the upper plate; L = length of the glass plate, and T= time (s).

Stability studies

Stability tests were performed for selected samples at 4.0±0.5°C (refrigerated temperature), room temperature, 27.0 ± 2.0^{0}C, and 37.0 ± 0.1^{0}C, (60% RH). Organoleptic properties, texture (feel), pH, viscosity and spreadability, of the formulations were noted at various intervals for a period of 30 days.

In vivo anti-inflammatory studies using carrageenan-Induced Acute Inflammatory Model

Twenty-four male Wistar rats (150 - 205g weight) were obtained from the animal house of the Faculty of Veterinary Medicine University of Ibadan. The rats were fed with rodent laboratory chow *ad libitum* and had free access to water. The experimental design was approved by the Institutional Animal Care and Use Research Ethics Committee (ACUREC) of the University of Ibadan (NHREC/UIACUREC/05/12/2022A) and conducted according to the NIH Guide for Care and Use of Laboratory Animals.

Carrageenan-induced edema in hind paws of Wistar rats was used to evaluate the topical anti-inflammatory effect of selected cream preparations. The solution of carrageenan in normal saline (1%w/v) was freshly prepared, immediately before the experiments. Edema was induced by intraplantar injection of 0.05 mL carrageenan solution into the right-hind paws of each animal of all groups except for Group A. Diclofenac cream (1.0%w/w) was used as an anti-inflammatory reference drug. The rats were randomly assigned to the following groups:
Group A (normal saline as negative control); Group B (Aqueous cream BP base); Group C (6.0% w/w turmeric cream); Group D (6.0%w/w turmeric and activated charcoal cream at ratio 2:1); Group E (6.0%w/w turmeric and activated charcoal cream at ratio 3:1); Group F (6.0%w/w Activated charcoal cream); Group G (marketed sample); Group H (1.0%w/w diclofenac cream) as the positive control.

All preparations (1.0g) were administered half an hour before the injection of carrageenan (1.0%w/v, 0.05 mL) in the intraplantar surface of the right hind paw and it was gently rubbed 50 times with the index finger. Diclofenac cream (1.0%), formulated creams, and marketed sample were applied in a similar way. The basal value (0 h) was measured by a plethysmometer (Ugo Basile, Milan, Italy) prior to the intraplantar injection of carrageenan and at 1.0, 2.0, and 3.0 h post-carrageenan injection. At the end of the experiment, the Wistar rats were euthanized and subplantar tissue of the carrageenan-injected paw was dissected for histological examination[23].

Statistical Analysis

Statistical analysis was carried out using the two-way analysis of variance (ANOVA) on a computer software GraphPad Prism© 4 (Graphpad Software Inc. San Diego, CA, USA). At 95% confidence interval, probability, p values less than or equal to 0.05 were considered significant.

RESULTS AND DISCUSSIONS

Phytochemical analysis of turmeric and activated charcoal powders

The results of phytochemical analysis of turmeric powder are presented in Table 2. The presence of flavonoids, alkaloids, saponin, and cardiac glycosides were confirmed in turmeric powder. The turmeric powder was devoid of anthraquinone. Flavonoids have been used extensively as anti-microbial, antiviral, anti-malarial, antioxidant, neuroprotective, antitumor, and anti-proliferative agents[24,25].

Therapeutically, alkaloids are particularly well known as anaesthetics, cardioprotective, and anti-inflammatory agents[26]. Biological applications of specific saponins include their uses as anti-inflammatory and as immune stimulating agents[27,28]. Tannins have been reported to possess antioxidant, anti-microbial and cardio-protective properties[29]. The results of phytochemical analysis of the activated charcoal powder showed the absence of alkaloids, anthraquinones, flavonoids and tannins, cardiac glycoside and saponins.

Physicochemical properties of turmeric and activated charcoal powders

The results of the physicochemical properties of the powders are presented in Table 3. Particle size of activated charcoal powder particles was 102±9.12µm while that for turmeric powder particles was 270±7.55µm. Turmeric powder had significantly ($p = 0.000$) larger particles than activated charcoal powder. The fine particle size of activated charcoal is related to its adsorption capacity and large surface area[30]. The pH of activated charcoal powder was 5.53±0.10 while that of turmeric powder was 6.20 ±1.05; both values can be considered near to the pH of the skin which ranges from 4.1 to 5.8[31]. The pH of topical formulations must be compatible with the pH of the skin where the formulation will be administered. Thus, incorporating the two powders into a cream base is not expected to adversely affect the pH balance of the skin. The swelling index of turmeric powder was significantly higher ($p = 0.000$) than that of activated charcoal. This may facilitate humectant effect when turmeric is incorporated into a cream formulation, having shown the capacity to retain moisture.

Particle, bulk and tapped densities for activated charcoal powder were higher than those of turmeric powder with larger particles. The decrease in density with larger particles may be attributed to the poor contact area (reduction in cohesiveness) between the particles causing increased volume and, accordingly, decreased density[32]. Carr's index was derived from the bulk and tapped densities of the powder. It is a measure of compressibility of a powder and provides an indirect measure of a material fluidity. The lower the Carr's index, the better the flowability, but the poorer the compressibility[33]. The result showed that turmeric powder had better flowability. The angle of repose of turmeric powder was also lower than that of activated charcoal, even though the value indicated fair flow property ($>30^o < 40^o$).

Table 1: Formulations of batches of creams containing activated charcoal and turmeric powders (made up to 100g in aqueous cream)

Batch	Cream formulation	Ingredients/ quantity (g)	
Aa	2% w/w Activated charcoal cream	Activated charcoal powder	2.0
Ab	3% w/w Activated charcoal cream	Activated charcoal powder	3.0
Ac	4% w/w Activated charcoal cream	Activated charcoal powder	4.0
Ad	5% w/w Activated charcoal cream	Activated charcoal powder	5.0
Ae	6% w/w Activated charcoal cream	Activated charcoal powder	6.0
Ba	2% w/w Turmeric cream	Turmeric powder	2.0
Bb	3% w/w Turmeric cream	Turmeric powder	3.0
Bc	4% w/w Turmeric cream	Turmeric powder	4.0
Bd	5% w/w Turmeric cream	Turmeric powder	5.0
Be	6% w/w Turmeric cream	Turmeric powder	6.0
Ca	2% w/w Turmeric and activated charcoal cream (2:1)	Turmeric powder Activated charcoal powder	1.33 0.67
Cb	3 % w/w Turmeric and activated charcoal cream (2:1)	Turmeric powder Activated charcoal powder	2.0 1.0
Cc	4 % w/w Turmeric and activated charcoal cream (2:1)	Turmeric powder Activated charcoal powder	2.67 1.33
Cd	5 % w/w Turmeric and activated charcoal cream ratio (2:1)	Turmeric powder Activated charcoal powder	3.33 1.67
Ce	6 % w/w Turmeric and activated charcoal cream (2:1)	Turmeric powder Activated charcoal powder	4.02 1.98
Da	2% w/w Turmeric and activated charcoal cream (3:1)	Turmeric powder Activated charcoal powder	1.50 0.50
Db	3% w/w Turmeric and activated charcoal cream (3:1)	Turmeric powder Activated charcoal powder	2.25 0.75
Dc	4% w/w Turmeric and activated charcoal cream (3:1)	Turmeric powder Activated charcoal powder	3.0 1.0
Dd	5% w/w Turmeric and activated charcoal cream (3:1)	Turmeric powder Activated charcoal powder	3.75 1.25
De	6 % w/w Turmeric and activated charcoal cream (3:1)	Turmeric powder Activated charcoal powder	4.5 1.5

Adsorption capacity of activated charcoal powder

The adsorption capacity of activated charcoal was 95.0%, attributable to micropores which increase surface area and enhances its adsorptive properties enabling it trap toxins and chemicals on its surface[34].

Antimicrobial activity of turmeric and activated charcoal powders

The zone of inhibition test is a widely used method in the determination of antimicrobial activity[35]. The results of antimicrobial test are presented in Table 4. The results for turmeric

powder revealed larger zones of inhibition against *E. coli* (p =0.000), *B. subtilis* (p = 0.013), and *S. aureus* (p = 0.001) with values of 17, 16 and 16 respectively, in comparison to those of activated charcoal powder. This indicates that these bacteria are more sensitive to turmeric powder. Furthermore, activated charcoal powder had zero inhibition on *Candida albicans*, *Aspergillus niger*, *Rhizopus stolonifera* and *Penicillin notatum*. The zones of inhibition against the fungi further confirmed their significantly higher sensitivity (p = 0.000) to turmeric powder.

Characterization of cream formulations

Organoleptic properties

The color of the cream formulations containing turmeric alone was yellow, while that of the creams containing activated charcoal alone was dark gray. Combination of both ingredients in the aqueous cream formulations produced a greenish gray color. The texture was smooth and not gritty on the skin. Creams containing 4 - 6%w/w turmeric alone had a slight pungent smell, characteristic of turmeric rhizome, while other cream formulations gave no specific odour.

Antioxidant activities of cream

Antioxidants are compounds that inhibit oxidation (usually occurring as autoxidation), a chemical reaction that can produce free radicals[36]. The results of antioxidant activity are presented in Table 5. Results showed that out of the five different concentrations of the formulated creams containing activated charcoal alone, batch Ac, containing 4%w/w activated charcoal, had the highest antioxidant activity of 57.81±0.11% which is indicative of its ability to scavenge oxygen molecules. On the other hand, formulated creams containing turmeric alone at concentrations 2.0 to 6.0%w/w showed antioxidant properties with values ranging from 68.98±0.21 to 82.48±0.11% that are significantly higher (p=0.000) than the antioxidant activity of aqueous cream alone or the marketed sample. Results for the creams containing the blend of both organic powders revealed that the antioxidant activity increased with the ratio of turmeric: activated charcoal. For the cream formulations containing 2.0 to 6.0%w/w of the blend at ratio 3:1, there was significantly higher (p = 0.001) antioxidant activity when compared with the aqueous cream base or the marketed sample. However, creams containing the blend of turmeric and activated charcoal powders with lower ratio 2:1 concentration (2.0 to 4.0%w/w) had lower antioxidant activity when compared to the marketed sample. Curcumin, through its chemical structure and the presence of hydroxyl and methoxy groups, is attributed to the antioxidant property as well as other properties of turmeric such as antimicrobial, and anti-inflammatory[37]. The high antioxidant values exhibited by these batches of formulations containing the blend of organic powders at ratio 3:1 suggest a synergistic effect.

***In vitro* antimicrobial activity**

The result of the antimicrobial activity revealed that the test microorganisms were most sensitive to cream formulations containing 6.0%w/w of activated charcoal alone (Ae), turmeric alone (Be), 6.0%w/w blends of both powders at ratios 2:1 (Ce) and 3:1 (De). The zones of inhibition of the creams for *B subtillis* ranged from 12 to 24 mm. The largest zones were observed for Be, 6% turmeric; Cd, 5% blend of turmeric and charcoal (2:1); Dd, 5% blend of turmeric and charcoal (31); De, 6% turmeric and charcoal (3:1) at 23, 22, 24 and 23 mm, respectively. The positive control, marketed sample and negative control had the zones of inhibition of 38, 27 and 10 mm, respectively. The least active formulations (zone of inhibition < 14 mm) are Aa, Ba, Ca and Da containing 2% charcoal, 2% turmeric, 2% blend of turmeric and charcoal (2:1) and 2% blend of turmeric and charcoal (3:1), respectively. The zones of inhibition increased significantly ($p<0.05$) with the ratio of turmeric: activated charcoal. Generally, the highest zones of inhibitions were obtained against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Cream formulation De, containing 6%w/w of turmeric and activated charcoal (ratio 3:1) gave the largest zones of inhibition (18- 26 mm) against all ten organisms in comparison to the other cream formulations.

The test microorganisms were less sensitive to creams containing lower concentrations 2.0 to 4.0%w/w of the organic powders either alone or blends. The controls, gentamicin and tioconazole (for bacteria and fungi, respectively), had zones of inhibition that were generally higher ($p>0.05$) than those of the cream formulations and the marketed sample. Cream formulation De, containing 6% turmeric powder and activated charcoal (3.1), showed promising antimicrobial activity against some Gram-positive and Gram-negative bacteria and fungi.

pH, viscosity and spreadability of the cream formulations

The barrier function, moisture retention, and microbial habitat of the skin are influenced by pH The pH plays a pivotal role in ensuring the effectiveness and safety of topical formulations including creams. Therefore, it is important to formulate creams with the correct pH level to prevent disruptions in the skin's natural balance and prevent skin irritation and dryness. The pH of the cream formulations containing the organic powders ranged from 5.9 to 7.1 and can be considered compatible with that of the skin[31].

The formulated creams had high viscosity suggesting their ability to adhere to the skin surface. Creams containing blends of the two organic powders had higher viscosity ($p = 0.05$) than those that contained the aqueous cream base, marketed sample or each powder alone. Viscosity also significantly increased ($p = 0.017$) with ratio of turmeric: activated charcoal. Furthermore, it was observed that the viscosity of all creams reduced with speed showing they exhibited pseudoplastic behavior. Many cosmetic creams are known to possess shear thinning behavior with viscosity values that depends on the shear intensity[38].

The efficacy of topical formulations such as creams highly depends on the patient spreading the formulation in an even layer on the skin to deliver a standard dose. Spreadability plays an important role in the ease of application, delivery of a standard dose of a medicated formulation to the skin and enhances efficacy of topical therapy[39,40]. Spreadability is the net result of a combination of rheological contributions, of which viscosity is included, in addition to the structural and viscoelastic characteristics that describe the rigidity, strength and relative contributions of elastic and viscous behaviour. The result of spreadability showed that the time taken for the cream to spread was 29 – 50 s, i.e., less than 1.0 min, indicative of good spread.

Table 3: Material properties of turmeric and activated charcoal powders (n = 3)

Properties	Turmeric powder (mean ± sd)	Activated charcoal powder (mean ± sd)
Particle size µm	270.00 ± 7.55	102.00 ± 9.12
pH	6.20 ±1.05	5.58 ± 0.05
Swelling index %	67.96 ± 0.33	38.80 ± 0.37
Particle density gcm^{-3}	0.858 ± 0.010	1.337 ± 0.083
Bulk density gcm^{-3}	0.506 ± 0.004	0.623 ± 0.033
Tapped density gcm^{-3}	0.610 ± 0.120	0.821±0.037
Carr's Index %	17.05 ± 5.80	24.11±0.20
Angle of repose ^{o}	39.00 ± 1.00	44.00 ±1.70

Table 4: Antimicrobial activities of turmeric and activated charcoal powders

Test organisms	Zone of inhibition (mm)	
	Activated charcoal	Turmeric powder
Staphylococcus aureus	14	16
Bacillus subtilis	12	16
Escherichia coli	12	17
Pseudomonas aeruginosa	10	13
Salmonella typhi	10	12
Klebsiellae pneumonia	10	10
Candida albicans	-----	14
Aspergillus niger	-----	12
Rhizopus stolonifer	------	12
Penicillin notatum	------	10

Table 5: Antioxidant activities of creams using DPPH assay

Formulation	Content	% DPPH inhibition
Aa	2% activated charcoal cream	31.94±0.02
Ab	3% activated charcoal cream	32.17±0.12
Ac	4% activated charcoal cream	57.81±1.11
Ad	5% activated charcoal cream	33.59±2.10
Ae	6% activated charcoal cream	37.36±3.13
Ba	2% turmeric cream	68.80±2.16
Bb	3% turmeric cream	79.57±4.11
Bc	4% turmeric cream	81.61±6.11
Bd	5% turmeric cream	82.38±0.10
Be	6% turmeric cream	82.48±1.11
Ca	2% turmeric and activated charcoal cream ratio 2:1	43.14±2.11
Cb	3% turmeric and activated charcoal cream ratio 2:1	58.98±3.21
Cc	4% turmeric and activated charcoal cream ratio 2:1	60.76±2.11
Cd	5% turmeric and activated charcoal cream ratio 2:1	74.76±4.11
Ce	6% turmeric and activated charcoal cream ratio 2:1	81.28±6.14
Da	2% turmeric and activated charcoal cream ratio 3:1	77.83±4.24
Db	3% turmeric and activated charcoal cream ratio 3:1	75.34±2.25
Dc	4% turmeric and activated charcoal cream ratio 3:1	86.77±2.14
Dd	5% turmeric and activated charcoal cream ratio 3:1	93.35±0.15
De	6% turmeric and activated charcoal cream ratio 3:1	96.67±4.15
E	Aqueous cream	57.73±3.43
F	Marketed sample anti-aging cream	62.88±3.14

Stability studies

No changes in the physical, pH and organoleptic properties of the creams throughout the period of storage at 4.0±0.5^{0}C and 27.0 ± 2.0^{0}C, and 37.0 ±0.1^{0}C, RH 60%. The cream formulations retained the properties of good spreadability and satisfactory consistency with no evidence of phase separation.

***In-vitro* anti-inflammatory activity**

Carrageenan-induced paw edema is an animal model suitable for evaluating inhibition of

edema. Biphasic edema induced by carrageenan involves the release of serotonin and histamine from mast cells in the first phase (within the first hour) and the mediation by prostaglandins, cyclooxygenase products in the second phase (after the first hour)[41].

The changes in paw edema1h, 2h and 3h after carrageenan administration (0.05mL of 1% intraplantar injection) are presented in Figure 1. The results showed that Group A (normal saline group) with basal value of 1.57 did not show any form of reduction in paw oedema after the 3h treatment. This group served as the negative control as no carrageenan was injected into their hind paws. Group B rats pretreated with carrageenan only, showed paw edema volume increased in a time-dependent manner after 1 h of induction, and reached its maximum at the third hour of monitoring. Group F containing 6%w/w activated charcoal alone also showed an increase in paw edema volume till time 3h. On the other hand, Group C pretreated with 6%w/w turmeric cream formulation showed reduction in paw edema volume when compared to Group B (carrageenan alone) at the 1st, 2nd, and 3rd hour of time interval after induction of edema. Groups D (6%w/w turmeric with activated charcoal 2:1) and E (6%w/w turmeric and activated charcoal 3:1) showed significant reduction ($p = 0.000$) in paw volume when compared to Groups B (carrageenan alone), C (6%w/w turmeric cream) and F (6%w/w activated charcoal cream) at all-time intervals. Group G (marketed sample) also showed significant ($p = 0.000$) anti-inflammatory effect when compared to Group B and those of other groups particularly at time $t = 3h$. The highest inhibition of inflammation was observed for Group H (diclofenac cream), the positive control, which showed a remarkable reduction in paw oedema. This is expected as diclofenac is a non-steroidal anti-inflammatory drug that reduces inflammation through the prostaglandin pathway[42]. Group E (6%w/w turmeric and activated charcoal 3:1) showed the highest inhibition of inflammation amongst the four groups pretreated with the test cream formulations containing either one or both organic compounds (Groups C, D, E and F). Figure 2(a) shows the paw edema damage of one of the pretreated groups of rats, Group B (carrageenan alone) at the 4th hour after the induction of edema. Carrageenan injection into the rat right hind paw caused massive accumulation of infiltrated inflammatory cells and edema formation with predominantly polymorphonuclear leukocytes (neutrophils) present. Figure 2(b) showed the infiltration of inflammatory cells and edema were significantly decreased following treatment with 6%w/w turmeric and activated charcoal cream 3:1 (Group E), and therefore supports the results presented.

CONCLUSION

Turmeric powder was confirmed to contain flavonoids, alkaloids and saponins which explain its potential as an antioxidant and antimicrobial agent. Adsorbance activity of activated charcoal powder was 95%. Turmeric and activated charcoal powders were successfully formulated into Aqueous cream BP at different concentrations each alone and blended at ratios 2:1 and 3:1. Incorporating a combination of turmeric powder and activated charcoal powder into aqueous cream base at concentrations 6%w/w and at ratio 3:1 gave satisfactory antioxidant and anti-inflammatory properties with promising antimicrobial activity against some Gram-positive and Gram-negative bacteria and certain fungi. This study reveals the potential of turmeric and activated charcoal powders in the development of cheaper, and effective cream formulations that can use their adsorbent, antioxidant, antimicrobial, and anti-inflammatory properties for retarding skin aging, and treating wounds, acne, and dermatitis. The natural therapeutic benefits of these ingredients can be utilized to provide a safer, alternative treatment option for skin health, potentially reducing reliance on conventional drugs like corticosteroids and synthetic antimicrobial agents.

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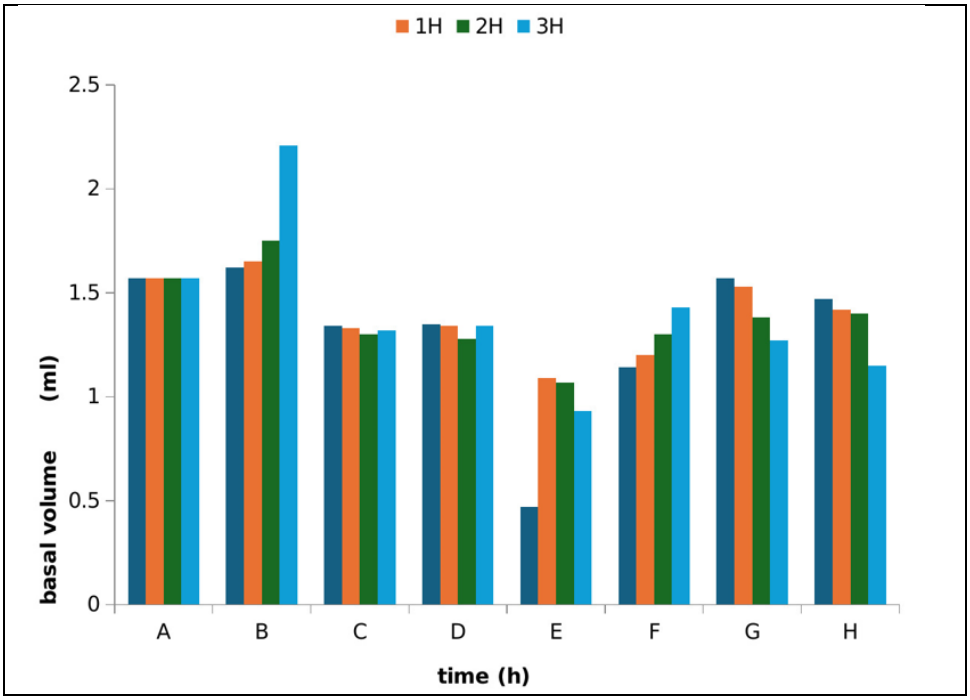

Figure 1: Plot of basal volume vs time (h)

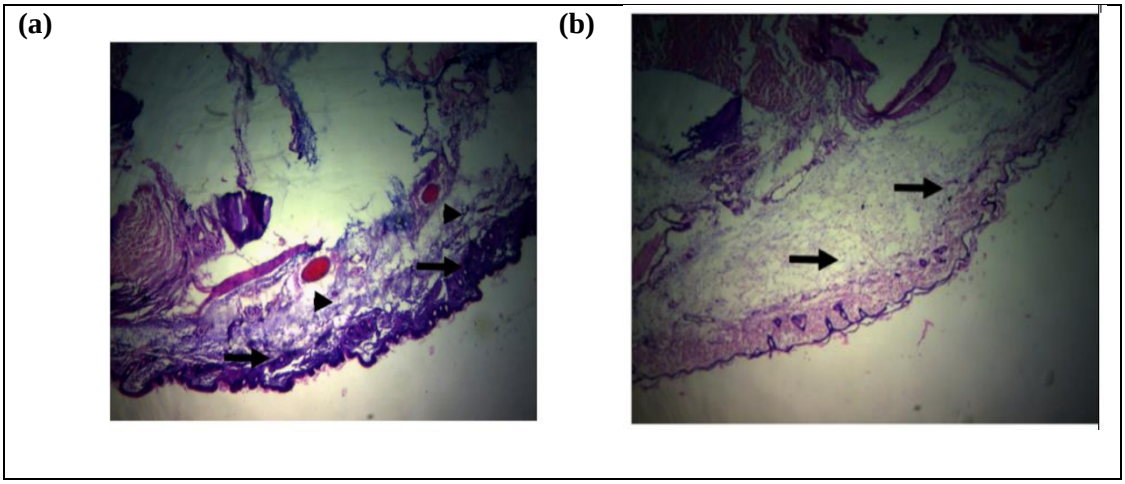

Figure 2: Photomicrographs of the hind paw sections of Wistar rats: (a) showing the histological damage and (b) reduced damage after administration of 6%w/w turmeric: activated charcoal cream (3:1)

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