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Pharmacological activities, chemical profile, and physicochemical properties of raw and commercial honey

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ABSTRACT

This study was designed to evaluate and correlate the pharmacological, phytochemical, and physicochemical properties of raw unifloral Mauritian eucalyptus honey (EH) and a commercially available honey (CH). The pharmacological activity was evaluated in terms of antibacterial, antioxidant (nitric oxide scavenging), anti-elastase, antityrosinase, antimelanogenic, and anticancer activity (MCF-7 and HeLa cell toxicity). The presence of phytochemicals including alkaloids, flavonoids, saponins, phenols, anthraquinones, and steroids were determined along with the total phenolic (TPC), total flavonoid (TFC), and tannin content (TC). Physicochemical properties including the pH, colour, total soluble solids, and density were also investigated. The results showed that EH displayed greater antibacterial, antioxidant, and anticancer activity against the MCF-7 cell line compared to CH, which also showed higher extracellular antimelanogenic activity. MH (IC50 = 532.75 µg/ml) displayed significantly greater scavenging activity than CH (IC50 = 647.6 µg/ml). To conclude, honey may be potentially exploited as complementary and alternative therapies for the management of infectious and chronic diseases.

1. Introduction

The global prevalence of infectious diseases, including bacterial infections, coupled to antibiotic resistance, has become a major public health burden, resulting in prolonged illness, disability, and death (WHO, 2016a). An estimate of more than 2 million infections and 23,000 deaths are attributable to antibiotic resistance annually in the United States while in Europe, antibiotic-resistant infections are estimated to cause 25,000 deaths (Gelband et al., 2015). More importantly, an alarming increase in death from chronic noncommunicable diseases, collectively responsible for about 70% of all deaths worldwide, has been noted (WHO, 2017a). Many of these diseases such as cardiovascular, cancer, diabetes, and chronic respiratory disorders are linked with an increase in oxidative stress caused by an imbalance between excess free radical production and endogenous antioxidant levels in the body (Pham-Huy et al., 2008). Amongst these, cancer is the second leading cause of death worldwide and was responsible for 8.8 million deaths in 2015 (WHO, 2017b). The most frequent type of cancer among women is breast cancer, affecting over 1.5 million women annually, with an associated mortality of 570,000 in 2015 (WHO, 2017c). On the other hand, cervical cancer ranks fourth in women with an estimated 270,000 deaths in 2012 (WHO, 2016b).

The antioxidant properties of natural products have received much attention of the scientific community to boost antioxidant defence mechanisms and a key strategy to curb down harmful free radicals. Reports tend to advocate the importance of antioxidants from natural products in oxidative stress, a major component of the onset and progression of a plethora of communicable and non-communicable diseases such diabetes and cancer (Mollica et al., 2017). On the other hand, enzyme inhibition in drug discovery has become a fundamental approach in pharmacology for the treatment of non-communicable diseases. For instance, tyrosinase is a key enzyme involved in melanin

Abbreviations: CE, Catechin equivalent; GAE, Gallic acid equivalent; MH, Mauritian Eucalyptus honey; RE, Rutin equivalent; CH, Commercial honey; TC, Tannin content; TFC, Total flavonoid content; TPC, Total phenolic content; TSS, Total soluble solids

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biosynthesis. Melanin is responsible for skin pigmentation and prevent UV-induced skin damage by absorbing UV rays and removing reactive oxygen species. However, excessive melanin formation and accumulation in the skin may cause hyperpigmentation disorders such as melasma, freckles, lentigines, and geriatric pigment spots (Aumeeruddy et al., 2018; Ya et al., 2015). In addition, loss of skin elasticity; one of the classical aging characteristics, is associated with an increase in enzymatic activity, particularly elastase, which breaks down elastin, a constituent of the connective tissue responsible for skin firmness and elasticity (Mathen et al., 2014). Furthermore, an increase in elastase activity is associated with several diseases such as rheumatoid arthritis, cystic fibrosis, chronic obstructive airway disease, psoriasis, and delayed wound healing (Siedle et al., 2003, 2007).

Recently, there has been a renewed interest in the role of natural products in drug discovery and development due to their low cost and because of the side effects associated with synthetic drugs. Honey is a natural product made by bees from nectar through a process of regurgitation and evaporation which is then stored in wax honeycombs (Khan et al., 2014). Honey has been used traditionally in different systems of medicine and has been found to possess extensive pharmacological properties including antimicrobial (Anyanwu, 2012; Mahendran and Kumarasamy, 2015), antioxidant (Alzahrani et al., 2012), antiinflammatory, analgesic (Alzubier and Okechukwu, 2011), hypoglycemic, hypolipidemic (Asaduzzaman et al., 2016), antihypertensive (Erejuwa et al., 2012), antiosteoporosis (Zaid et al., 2012), immunomodulatory, wound healing (Majtan, 2014), cardio-protective properties, among others (Khalil et al., 2015).

Mauritius is a tropical island in the southwest of the Indian Ocean, with a population of 1,222,217 according to latest estimates recorded in 2017. The island is 61 km long, 47 km wide, with a total surface area of 1865 km^2, and is located 800 km east of Madagascar (Mahomoodally and Aumeeruddy, 2017; Mahomoodally and Sreekeesoon, 2014). The main melliferous plants in Mauritius are Longan, Tamarind, Wild pepper, Campeche, Litchi, and Eucalyptus. However, due to loss of interest by apiarists, Mauritius is not self-sufficient in the production of honey and hence imports honey from different countries (Kinoo et al., 2012). Previous studies have probed into the antimicrobial and antioxidant potential of Mauritian Wild pepper (*Schinus terebinthilolius*), Litchi (*Litchi chinensis*), and Longan (*Dimocarpus longan*) (Dor and Mahomoodally, 2014; Kinoo et al., 2012). However, to the best of our knowledge, local Eucalyptus honey has not been explored for any potential pharmacological activity. In addition, due to variations observed in studies regarding geographical origin, climatic condition, floral source, and storage conditions, comparative studies investigating different honey samples is important to understand the factors responsible for these variations in order to obtain a better medicinal product for therapeutic use. In this context, the present study aimed to compare the antibacterial, antioxidant, antielastase, antityrosinase, antimelanogenic, and anticancer activity of raw Mauritian Eucalyptus honey and a commercial honey in relation to their phytochemical composition and physicochemical properties.

2. Materials and methods

2.1. Reagents

All chemicals and reagents used in the study were of analytical grade and were purchased from reliable firms and institutes. Porcine pancreatic elastase type IV, N-succinyl-(Ala)3-p-nitroanilide, Trizma base, XTT cell proliferation kit II, L-ascorbic acid, Actinomycin D, mushroom tyrosinase, L-tyrosine, and kojic acid were obtained from Sigma Aldrich, MO, USA. The human cervical adenocarcinoma (HeLa) and human breast adenocarcinoma (MCF-7) cell lines were obtained from the European Collection of Cell Cultures (ECACC, England, UK). Minimum Essential Medium (MEM), trypsin-EDTA, fetal bovine serum (FBS), phosphate buffer saline (PBS), Mueller-Hinton agar (MHA), Mueller-Hinton broth (MHB), and antibiotics were supplied by Thermofisher scientific (Modderfontein, Johannesburg, RSA). Sodium nitroprusside and Griess-Ilosvsy's nitrite reagent were purchased from Merck Millipore, Darmstadt, Germany.

2.2. Materials

Two honey samples: (i) raw (unprocessed) unifloral Mauritian eucalyptus (*Eucalyptus* sp.) honey (MH), obtained from the Entomology division, Reduit, Mauritius and (ii) commercial honey, a commercially available honey (CH), labelled “natural honey” with no specification of floral source, was purchased from a local shop in Mauritius. Following collection, the honey samples were stored at room temperature in the dark during the whole period of the study.

2.3. Antibacterial assays

Disc and well diffusion methods were performed following the guidelines of “The Clinical and Laboratory Standards Institute (CLSI)” (CLSI, 2015). The two assays were carried out in parallel and parameters including inoculum level, depth of agar, and size of disc and well, were kept constant. Measurements were carried out in triplicate.

2.3.1. Bacteria used

Clinical isolates including *Proteus* sp., *Klebsiella* sp., *Streptococcus* sp., *Pseudomonas* sp., and *Escherichia coli* were obtained from Biosante Laboratory, Mauritius and from Victoria Hospital, Candos, Mauritius, while American Type Culture Collection (ATCC) strains including *Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* ATCC 35984, and *Staphylococcus epidermidis* ATCC 14990 were obtained from the Department of Health Sciences, Faculty of Science, and Department of Agricultural and Food Science, Faculty of Agriculture, University of Mauritius. All strains were sub-cultured on Mueller-Hinton Agar (MHA) and grown in Mueller-Hinton broth (MHB) at 37 °C prior to the day of use.

2.3.2. Disc diffusion assay

One hundred microlitres of bacterial culture, adjusted to 0.5 McFarland standard turbidity scale in MHB, was spread evenly on the surface of MHA plates. Paper discs (5 mm), prepared from Whatmann filter paper, were impregnated with 30 µl of honey (undiluted), and placed on the inoculated plates. Discs impregnated with 30 µl of streptomycin, cloxacillin, ampicillin, and chloramphenicol, at 1 mg/ml, were used as positive controls, while sterile distilled water was used as the negative control. The plates were incubated at 37 °C for 24 h and the diameter of the zone of inhibition (ZOI) including that of the discs were measured in mm.

2.3.3. Well diffusion assay

One hundred microlitres of bacterial culture, adjusted to 0.5 McFarland standard turbidity scale in sterile MHB, was spread evenly on the surface of MHA plates. Five millimeter diameter wells, sufficiently spaced to avoid overlapping of results, were punched into the surface of the agar using a sterile cork borer followed by addition of 30 µl of honey (undiluted) to each well. Four antibiotics were used as positive controls including streptomycin, cloxacillin, ampicillin, and chloramphenicol, at a concentration of 1 mg/ml, while sterile distilled water was used as negative control. The plates were incubated at 37 °C for 24 h and the diameter of ZOI including that of the well were measured in mm.

2.4. Antioxidant assay

2.4.1. Nitric oxide scavenging assay

The nitric oxide (NO) scavenging activity of the samples was

measured according to the method described by (Mayur et al., 2010). The honey samples were prepared by dissolving the honey in ethanol to a starting concentration of 10 mg/ml. To the top row of a 96-well plate, 20 µl of distilled water and 80 µl of the honey sample were added. The honey samples were double diluted to a final concentration ranging from 2000 µg/ml to 15.6 µg/ml. Sodium nitroprusside (10 mM, 50 µl) was added to all the wells followed by incubation at room temperature under light for 90 min. After incubation, Griess-Ilosvsy's nitrite reagent (100 µl) was added to the test wells and distilled water to the colour control wells. The nitrite content was measured at 546 nm after a 5 min incubation in the dark. L-ascorbic acid (10 mg/ml) and ethanol was used as the positive and negative controls, respectively. The radical scavenging activity was determined as percentage NO radical-scavenging activity which was calculated by the equation: % NO radical-scavenging = [(AC – AS)/AC] × 100; where AC is the absorbance of the control solution that contains only NO, and AS is the absorbance of the honey samples in NO solution. From these results, the fifty percent inhibitory concentration (IC_{50}) was determined using the GraphPad Prism 4.0 program (GraphPad Software, Inc., CA, USA).

2.5. Elastase inhibitory activity

The ability of the honey samples to inhibit porcine pancreatic elastase (PPE) was determined by measuring the release of p-nitroaniline from N-succinyl-(Ala)3-p-nitroanilide spectrophotometrically according to the method of Bieth et al. (1974) with slight modifications. The reaction mixture contained 100 mM Tris buffer (pH 8.0), 0.5 M HCl, and the test sample (honey and the positive drug control, ursolic acid) which were serially diluted to 250-3.13 µg/ml. PPE (5 mM, 20 µl) was then added and the reaction mixture was incubated for 15 min followed by the addition of N-succinyl-(Ala)3-p-nitroanilide (4 mM). A vehicle control where the sample was replaced by methanol was included as the 100% rate, and 0% where the enzyme and substrate were replaced, respectively, by buffer solution. The change in the absorbance of the reaction mixture was measured kinetically at 405 nm for 15 min using KC Junior software and a BIO-TEK Power-Wave XS multiwell plate reader (A.D.P, Weltevreden Park, South Africa). One unit of elastolytic activity is defined as the release of 1 µM of p-nitroaniline/min. The concentration of honey at which fifty percent of the enzyme was inhibited (IC_{50}) was then calculated.

2.6. Tyrosinase inhibitory activity

The antityrosinase assay was performed according to the method described by (Mapunya et al., 2018), with few modifications. The honey samples were dissolved in 100 µl DMSO to a 20 mg/ml stock solution which was diluted with 50 mM potassium phosphate buffer (pH 6.5). In a 96-well microtitre plate placed on ice, 30 µl of tyrosinase enzyme (333 units/ml in phosphate buffer pH 6.5) was added to 70 µl of varying concentrations of honey, in triplicate. After 5 min of incubation on ice, 110 µl of substrate (2 mM L-tyrosine) was added to all the wells. The final concentrations of each sample and positive control (kojic acid) ranged from 1000 to 1.5 µg/ml. The optical density (OD) was then measured over a period of 30 min at a wavelength of 492 nm using BIO-TEK power Wave XS multi-well plate reader and KC Junior software. The fifty percent inhibitory concentration (IC_{50}) was then determined.

2.7. Melanin inhibitory activity

2.7.1. B16F10 melanoma cell culture

Mouse melanocytes (B16F10) were cultured in complete Minimum Essential Eagle's Medium (MEM), containing 10% FBS, 1.5 g/L $NaHCO_3$, 2 mM L-glutamate, 10 mg/ml streptomycin, and 0.25 mg/ml fungizone.

2.7.2. Measurement of melanin production in cultured B16F10 melanoma cells

The inhibitory effect of honey on melanin production was determined following the Hill method previously described by Matsuda et al. (2004). The cultured B16F10 mouse melanoma cells were trypsinized (0.25% trypsin and 0.1% EDTA at 37 °C for 5–10 min) and plated into 24-well plates (5×10^4 cells/well in 1.5 ml of MEM). The plated cells were incubated for 24 h at 37 °C in the CO_2 incubator. Following incubation, 500 µl of each honey sample (concentration ranging from 500 to 15.6 µg/ml) was added to each well in duplicate, and the treated 24-well plates were incubated for 3 days at 37 °C in the CO_2 incubator. Test samples and theophylline (negative control) were dissolved in DMSO. The final concentration of DMSO was 5%. The untreated cells were used as the control group.

After incubation, the cultured medium was removed by a pipette and assayed for extracellular melanin as follows: The cultured medium was centrifuged (900 g, 20 min at 4 °C) to separate the cellular components and extracellular components. One millilitre of a mixture of 0.4 M Tris buffer (pH 6.8) and ethanol (9:1, v/v) was added to 1 ml of the supernatant. The OD of the resulting solution was measured at 475 nm, and the amount of extracellular melanin was determined.

To determine the intracellular melanin production, the remaining melanoma cells were washed with CMF-D-PBS (Calcium and Magnesium Free Dulbecco's-Phosphate Buffered Saline) and trypsinized (100 µl of 0.25% trypsin and 0.1% EDTA at 37 °C for 5–10 min). The cells were digested by the addition of 400 µl of 1 N NaOH and then left standing for 16 h at room temperature. The OD of the resulting solution was measured at 475 nm, and the amount of intracellular melanin was determined. Melanin inhibition was determined by comparing the OD of the dose dependant treated cells with the untreated cells and the IC_{50} values were determined.

2.8. Anticancer activity

2.8.1. Cell culture

The human breast adenocarcinoma (MCF-7) and human cervical (HeLa) cell lines were maintained in MEM supplemented with 10% FBS and 1% antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin) and 250 µg/ml fungizone. The cells were grown statically at 37 °C in a humidified incubator set at 5% CO_2. Once confluent, the cells were subcultured by treating them with trypsin-EDTA (0.25% trypsin containing 0.53 mM EDTA) for a maximum of 15 min.

2.8.2. MCF-7 and HeLa cell inhibition

The cytotoxicity of the honey samples were evaluated using the XTT cell proliferation Kit II according to the method of Zheng et al. (2001). MCF-7 and HeLa cells (1×10^5 cells/ml) were seeded in 96-well microtitre plates respectively and allowed to attach for 24 h at 37 °C and 5% CO_2. The honey samples were prepared at 20 mg/ml stock concentrations in DMSO. The cells were treated with honey at concentrations ranging from 400 to 3.13 µg/ml and the positive drug control, actinomycin D, with concentrations ranging between 0.5 µg/ml and 0.002 µg/ml. A vehicle control (2% DMSO) was included. The treated cells were further incubated for 72 h followed by the addition of 50 µl XTT to a final concentration of 0.3 mg/ml. The plates were incubated with the viability reagent for 2 h and the absorbance of the colour complex was measured at 490 nm with a reference wavelength set at 690 nm for XTT using KC Junior software and a BIO-TEK Power-Wave XS multi-well plate reader (A.D.P, Weltevreden Park, South Africa). The assay was performed in triplicate and the fifty percent inhibitory concentration (IC_{50}) values of the samples were calculated using the GraphPad Prism 4.0 program (GraphPad Software, Inc., CA, USA).

2.9. Phytochemical analysis

2.9.1. Qualitative phytochemical test

Phytochemical screening for the presence of alkaloids, flavonoids, saponins, phenols, anthraquinones, and steroids was performed using standard protocols (Andzouana and Mombouli, 2011; Tiwari et al., 2011).

2.9.2. Quantitative phytochemical test

Total phenolic content (TPC) was determined using the Folin–Ciocalteu method as described by Picot et al. (2014). Five hundred microlitres of test sample was mixed with 2500 µl Folin–Ciocalteu reagent (ten-fold diluted) and 2000 µl of sodium carbonate solution (7.5%). The mixture was allowed to stand for 30 min and the absorbance of the solution was measured spectrophotometrically at 760 nm. All determinations were performed in triplicate. TPC was expressed as µg of gallic acid equivalent (GAE) per g of sample (µg GAE/g sample).

Total flavonoid content (TFC) was determined according to the aluminum chloride colorimetric method as described previously (Picot et al., 2014; Mollica et al., 2017). The reaction mixture containing 2 ml of diluted sample and 2 ml of 2% $AlCl_3$ solution was allowed to stand at room temperature for 30 min after which the absorbance of the solution was measured spectrophotometrically at 420 nm. All determinations were performed in triplicate and TFC was expressed as µg of rutin equivalent (RE) per g of sample (µg RE/g sample).

Tannin content (TC) was measured using the vanillin-HCl method as described by Mak et al. (2013). Briefly, 1 ml of sample was mixed into 5 ml of reagent mixture (4% vanillin in methanol and 8% HCl in methanol in the ratio of 1:1). After 20 min, the resulting colour change was measured spectrophotometrically at 500 nm. TC was expressed as µg catechin equivalent (CE) per g sample (µg CE/g sample).

2.10. Physicochemical properties

Physicochemical properties including pH, colour, TSS, and density were tested. The pH was measured using a digital pH meter (Mettler Toledo™ FE20 FiveEasy™). For colour measurement, CIELAB L* a* b* colour parameters were determined using a chromameter (Minolta CR-410, Konica Minolta, Japan), which was placed directly over the samples in petri dishes filled to the brim. L* represents lightness, a* measures the degree of red (+a*) or green (-a*) colours and b* parameter indicates the degree of the yellow (+b*) or blue (-b*) colours (Boussaid et al., 2018). In addition, TSS was measured using a digital hand-held "Pocket" refractometer (ATAGO, PAL-3) with ranges of 0–93°Brix. Density was measured according to the method described by Kinoo et al. (2012) using the formula: Density = Mass of sample/volume of sample. All measurements were done in triplicate.

2.11. Statistical analysis

All data presented in this study were analysed using Microsoft Excel 2010, Minitab version 16, and GraphPad Prism 4.0. One way ANOVA (Tukey's test) was used for evaluation of significant differences between the variables. Pearson's correlation was used to evaluate correlation between the variables. $P < 0.05$ was considered as statistically significant.

3. Results and discussion

3.1. Antibacterial activity

The results of the antibacterial activity of honey are presented in Table 1 and Table 2. The bacterial strains displayed variation in susceptibility to the tested samples. In general, the ATCC strains were found to be more susceptible compared to the clinical isolates. We found that the two honey samples were most effective against *E. coli*. The *E. coli* clinical isolate was more susceptible to CH (ZOI = 24 mm) while MH showed greater activity against *E. coli* ATCC 25922 (ZOI = 28 mm). In fact, MH (ZOI = 8–11.7 mm) was found to be significantly more effective ($p < 0.05$) than CH (ZOI = 7–9.3 mm) against all other tested strains except *P. mirabilis* ATCC 12453, which was resistant to both honey samples. In addition, CH was also ineffective against *Streptococcus* sp. and both *S. epidermidis* strains (ATCC 35984 and ATCC 14990).

The antibacterial activity of honey has been reported to be mainly due to the (i) osmotic effect caused by its high sugar content, (ii) low pH, and (iii) hydrogen peroxide which is probably the main antibacterial compound although the non-peroxide constituents are also known to be important (Eteraf-Oskouei and Najafi, 2013). Normally, honey is subjected to thermal treatment before marketing to remove contaminants and delay crystallisation. It has been shown that even relatively mild heat processing can reduce its antimicrobial activity (Chen et al., 2012). However, the study of Pimentel-González et al., 2017 showed that thermal processing may either decrease or increase the antibacterial activity of honey depending on the honey's floral sources and the bacteria tested. This may explain the higher activity of CH against one out of ten bacterial strains tested in the present study but weakly active against the other tested bacteria compared to MH.

Comparison of the two antibacterial assays conducted revealed that the two honey samples showed variation, displaying greater ZOI in the well diffusion method against some tested bacteria including *E. coli* and *S. epidermidis*, while they showed greater ZOI against *P. aeruginosa* ATCC 27853 and *Klebsiella* sp. in the disc diffusion assay (Fig. 1). Overall, the variations observed were dependent on the bacteria tested and the type of honey. It is noteworthy that the variations observed among the two assays in the current study were also observed previously (Kinoo et al., 2012). However, the latter observed greater ZOI using well diffusion assay. In addition, Schneider et al. (2013) found that when using the disc diffusion assay, honey did not diffuse into the agar and remained on the surface of the disc, hence displaying no antibacterial effect. This discrepancy among the studies might be due to the variations in the viscosity and density of honey, or the presence of any high molecular weight antibacterial compounds which may not diffuse through the agar.

3.2. Antioxidant activity

The antioxidant activity of the honey samples in terms of their scavenging activity against NO radical are shown in Table 3. Among the tested samples, MH (IC_{50} = 532.75 µg/ml) displayed significantly greater scavenging activity than CH (IC_{50} = 647.6 µg/ml) ($p < 0.05$). However, the two honey samples were found to exhibit significantly low scavenging activity compared to the positive control, L-Ascorbic acid (IC_{50} = 66.4 µg/ml) ($p < 0.05$). Few studies have actually reported the NO scavenging activity of honey in comparison to other assays such as DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging and FRAP (ferric reducing antioxidant power). Compared to previous studies, the IC_{50} values of the honey samples tested in the present study were found to be higher (hence lower NO scavenging activity) compared to previous work (Ukkuru, 2015) (IC_{50} = 126–625.79 µg/ml).

The superiority of the antioxidant activity of raw honey over commercial honey, as observed in the present study, is in agreement with other studies (Kishore et al., 2011; Muruke, 2014). On the other hand (Dor and Mahomoodally, 2014), found that both processed and raw honeys of Mauritius and its neighboring Rodrigues island had approximately the same antioxidant activity, showing variations among different antioxidant assays, indicating the necessity to conduct more antioxidant assays. This discrepancy in the antioxidant activity of raw (unprocessed) and processed honey is probably due to the formation of melanoidin, an antioxidant product formed in the final stage of the Maillard reaction (Brudzynski and Miotto, 2011b). The effect of

Table 1
Antibacterial activity of undiluted samples using disc diffusion assay.

	MH	CH	Streptomycin	Ampicillin	Cloxacillin	Chloramphenicol
E.coli (clinical isolate)	22.3 ± 0.58^{bc}	24.0 ± 0.0^{a}	10.7 ± 0.58^{d}	NI	NI	21.0 ± 1.0^{c}
E.coli ATCC 25922	28.0 ± 1.0^{a}	25.3 ± 0.58^{b}	25.3 ± 0.58^{b}	13.0 ± 0.0^{c}	NI	25.7 ± 0.58^{b}
Proteus spp (clinical isolate)	11.7 ± 0.58^{c}	8.0 ± 1.0^{d}	18.7 ± 0.58^{a}	NI	NI	15.7 ± 0.58^{b}
P. mirabilis ATCC 12453	NI	NI	23.7 ± 0.58^{c}	20.0 ± 1.0^{d}	28.7 ± 0.58^{a}	26.7 ± 0.58^{b}
Pseudomonas spp (clinical isolate)	10.7 ± 0.58^{a}	8.7 ± 0.58^{b}	NI	NI	NI	NI
P. aeruginosa ATCC 27853	9.7 ± 0.58^{e}	9.3 ± 0.58^{e}	25.7 ± 0.58^{a}	15.3 ± 0.58^{c}	13.7 ± 0.58^{d}	23.0 ± 0.0^{b}
Klebsiella spp (clinical isolate)	11.7 ± 0.58^{b}	7.0 ± 0.0^{c}	16.3 ± 0.58^{a}	NI	NI	8.0 ± 0.0^{c}
Streptococcus spp (clinical isolate)	8.0 ± 0.0^{c}	NI	NI	16.0 ± 1.0^{b}	NI	21.0 ± 1.0^{a}
S. epidermidis ATCC 35984	10.0 ± 0.0^{c}	NI	NI	12.7 ± 0.58^{b}	9.0 ± 1.0^{c}	24.3 ± 0.58^{a}
S. epidermidis ATCC 14990	11.3 ± 0.58^{d}	NI	20.7 ± 0.58^{c}	19.7 ± 0.58^{c}	38.7 ± 0.58^{a}	23.7 ± 0.58^{b}

Note: Diameter of inhibition zones includes diameter of discs (5 mm); Values represent mean of triplicate ± standard deviation (n = 3) in mm; NI: No inhibition; MH: Mauritian Eucalyptus honey; CH: Commercial honey; All antibiotics were tested at 1 mg/ml; Different letter superscript between columns means significantly different ($p < 0.05$).

thermal treatment (during processing) on the antioxidant activity of honey depends on the initial concentration of melanoidins in unheated honeys. Brudzynski and Miotto (2011a) found that at low initial concentrations of melanoidins in light and medium honey, thermal treatment accelerated the formation of new melanoidins and increased the overall antioxidant activity. However, at higher initial concentrations in fractions of dark honeys, thermal treatment results in a decrease in the old pools of melanoidins hence reducing the antioxidant activity.

3.3. Elastase inhibitory activity

The elastase inhibitory activity of the two honey samples are presented in Table 3. No inhibition was observed at the highest concentration tested (250 µg/ml) in contrast to the positive control, ursolic acid, which displayed an IC_{50} value of 4.27 µg/ml. To the best of our knowledge, we found no previous study on the inhibitory activity of honey on the enzyme porcine pancreatic elastase. Nonetheless, *in silico* analysis revealed the efficacy of 12 constituents of honey to dock and bind with human neutrophil elastase, indicating its potential as elastase inhibitors (Narayanaswamy et al., 2015). Therefore, it is recommended that future studies explore the antielastase activity of more honey samples from different floral sources at a higher concentration than that tested in the present study.

3.4. Tyrosinase inhibitory activity

The inhibitory effect of honey on tyrosinase activity are displayed in Table 3. At the highest concentration tested (1000 µg/ml), no inhibitory activity was observed in contrast to the positive control kojic acid (IC_{50} = 2.849 µg/ml). Nonetheless, the tyrosinase inhibitory properties of honey has been demonstrated by other studies. For instance, honey originating from Sardinian Eucalyptus (*Eucalyptus* sp.) was found to inhibit the enzyme (IC_{50} = 157.7 mg/ml) (Di Petrillo et al., 2018). Another study (Jantakee and Tragoolpua, 2015) found that 16 tested honey samples of different floral sources exhibited potent anti-tyrosinase activity, with manuka honey and coffee honey displaying 88% and 63% inhibition, respectively. However, it is important to highlight that in their study, the highest tested concentration of honey (50%) is higher compared to that of the current study (1000 µg/ml = 10%) which indicates the necessity for further studies to explore the potential tyrosinase inhibitory activity of MH and CH at a higher concentration.

3.5. Melanin inhibitory activity

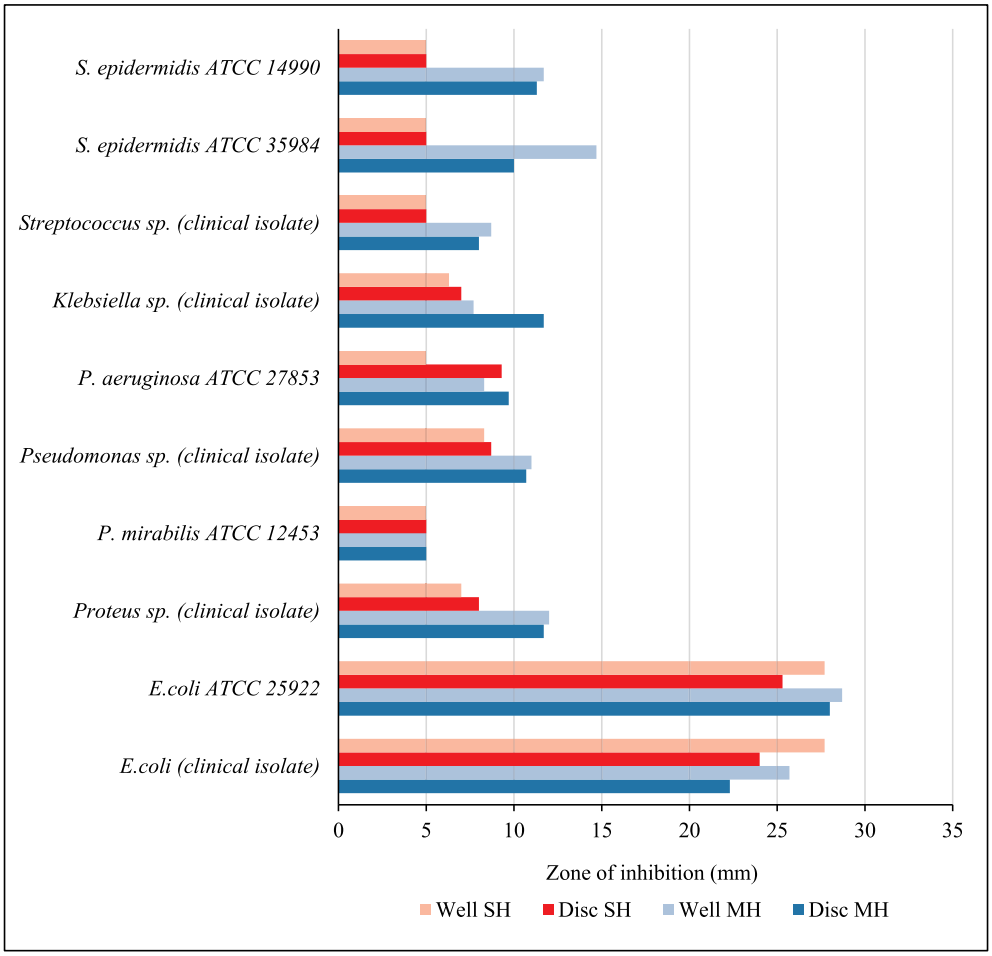
The IC_{50} values obtained in the melanin inhibition assay are shown in Table 3. We observed that honey displayed no inhibition on intracellular melanin synthesis at the highest tested concentration (500 µg/ml). In contrast, although MH did not inhibit extracellular melanogenesis at the highest tested concentration (500 µg/ml), a 50% inhibition was observed by sample CH at a concentration of 96.66 µg/ml, which was significantly more effective ($p < 0.05$) than the positive control arbutin (IC_{50} = 99.57 µg/ml). To the best of our knowledge, this is the first study to explore the potential of honey as a melanin inhibitor. However, it should be noted that the tyrosinase inhibitory activity of honey, as observed by other studies (Di Petrillo et al., 2018; Jantakee and Tragoolpua, 2015), indicates its indirect role as an anti-melanogenic agent since the enzyme tyrosinase is involved in melanogenesis (Aumeeruddy et al., 2018).

It is important to point out that honey is one of the most widely traditionally used product in the management of skin diseases (Ediriweera and Premarathna, 2012). Commercially, honey is a major ingredient in various cosmetic skin products indicating its potency in the above mentioned skin related bioactivies (antielastase,

Table 2
Antibacterial activity of undiluted samples using well diffusion assay.

	MH	CH	Streptomycin	Ampicillin	Cloxacillin	Chloramphenicol
E.coli (clinical isolate)	25.7 ± 0.58^{b}	27.7 ± 0.58^{a}	12.3 ± 0.58^{d}	NI	NI	18.3 ± 0.58^{c}
E.coli ATCC 25922	28.7 ± 0.58^{ab}	27.7 ± 0.58^{b}	28.3 ± 0.58^{ab}	16.3 ± 0.58^{c}	NI	29.7 ± 0.58^{a}
Proteus spp (clinical isolate)	12.0 ± 1.0^{c}	7.0 ± 0.0^{d}	21.7 ± 0.58^{a}	NI	NI	18.3 ± 0.58^{b}
P. mirabilis ATCC 12453	NI	NI	28.3 ± 0.58^{c}	29.3 ± 0.58^{bc}	30.0 ± 0.0^{ab}	31.3 ± 0.58^{a}
Pseudomonas spp (clinical isolate)	11.0 ± 1.0^{a}	8.3 ± 0.58^{b}	NI	NI	NI	NI
P. aeruginosa ATCC 27853	8.3 ± 0.58^{d}	NI	26.3 ± 0.58^{a}	17.7 ± 0.58^{c}	16.7 ± 0.58^{c}	24.7 ± 0.58^{b}
Klebsiella spp (clinical isolate)	7.7 ± 0.58^{c}	6.3 ± 0.58^{d}	17.0 ± 0.0^{a}	NI	NI	9.0 ± 0.0^{b}
Streptococcus spp (clinical isolate)	8.7 ± 0.58^{c}	NI	NI	22.3 ± 0.58^{b}	NI	24.7 ± 0.58^{a}
S. epidermidis ATCC 35984	14.7 ± 0.58^{b}	NI	NI	13.7 ± 0.58^{bc}	13.3 ± 0.58^{c}	30.3 ± 0.58^{a}
S. epidermidis ATCC 14990	11.7 ± 0.58^{c}	NI	23.3 ± 0.58^{c}	20.3 ± 0.58^{d}	41.3 ± 0.58^{a}	27.0 ± 1.0^{b}

Note: Diameter of inhibition zones includes diameter of wells (5 mm); Values represent mean of triplicate ± standard deviation (n = 3) in mm; NI: No inhibition; MH: Mauritian Eucalyptus honey; CH: Commercial honey; All antibiotics were tested at 1 mg/ml; Different letter superscript between columns means significantly different ($p < 0.05$).



Note: Diameter of inhibition zones includes diameter of disc and well (5 mm); ZOI of 5mm indicates no inhibition; MH: Mauritian Eucalyptus honey; CH: commercial honey

Fig. 1. Mean ZOI for disc diffusion and well diffusion assay.

antityrosinase, and antimelanogenic). Nonetheless, further in-depth studies are required in order to develop highly potent bio-products before its clinical use in the treatment of skin disorders.

3.6. Anticancer activity

As shown in Table 3, the two honey samples showed no toxicity toward the HeLa cell line at a highest tested concentration of 400 µg/ml. On the contrary, the study of (Fauzi et al., 2011) revealed the

Table 3
Other pharmacological activities of honey samples.

Samples	NO scavenging	Elastase inhibition	Tyrosinase inhibition	Melanin inhibition		Anticancer	
				Intracellular	Extracellular	MCF-7 cell line inhibition	HeLa cell line inhibition
	IC_{50} (µg/ml)						
MH	532.75 ± 3.6[a]	NI[A]	NI[B]	NI[C]	NI[C]	159.4 ± 3.6[b]	NI[D]
CH	647.6 ± 2.1[b]	NI[A]	NI[B]	NI[C]	96.66 ± 1.985[a]	NI[D]	NI[D]
L-Ascorbic acid	66.4 ± 1.9[c]	–	–	–	–	–	–
Ursolic acid	–	4.27 ± 0.65	–	–	–	–	–
Kojic acid	–	–	2.849 ± 4.469	–	–	–	–
Arbutin	–	–	–	99.57 ± 1.998	99.57 ± 1.998[b]		
Actinomycin D	–	–	–	–	–	0.0075 ± 3.9[a]	0.0022 ± 3.4[a]

Note: Values represent mean of triplicate ± standard deviation (n = 3) in µg/ml; MH: Raw Mauritian Eucalyptus honey; CH: Commercial honey; Different letter superscript (lowercase) within column means significantly different ($p < 0.05$).
-: Not tested.
IC_{50}: Fifty percent inhibitory concentration.
[a] : No inhibition at the highest concentration tested of 250 µg/ml.
[b] : No inhibition at the highest concentration tested of 1000 µg/ml.
[c] : No inhibition at the highest concentration tested of 500 µg/ml.
[d] : No inhibition at the highest concentration tested of 400 µg/ml.

anticancer activity of Malaysian tualang honey against HeLa cell line, with an EC_{50} doses of 2.4% v/v. Although CH honey displayed no anticancer activity on MCF-7 cell line at the highest concentration tested (400 µg/ml) in our study, MH was effective with an IC_{50} value of 159.4 µg/ml. Nonetheless, the positive control, actinomycin D (IC_{50} = 0.0075 µg/ml), showed higher activity compared to MH. Indeed, the cytotoxicity of various honey samples including Malaysian Tualang (Fauzi et al., 2011; Yaacob et al., 2013) and Acacia honey (Salleh et al., 2017), New Zealand Manuka honey (UMF 5+, 10+, 15+, 16+, 18+) (Portokalakis et al., 2016), Greek (Spilioti et al., 2014; Tsiapara et al., 2009), Turkish (Seyhan et al., 2017) and Indian honey (Jaganathan et al., 2010) from different floral sources against the MCF-7 cell line have been reported.

The cytotoxic effect of honey is attributed to its phenolic constituents. Chrysin is a major phenolic compound identified in honey and its toxic effect has been demonstrated against MCF-7 cells (Yang et al., 2013). It has also been found that honey exhibits its anticancer activity through several mechanisms depending on the floral sources, including its apoptotic, antiproliferative, antiinflammatory, immunomodulatory, antioxidant, antimutagenic, estrogenic modulatory activity, among others (Ahmed and Othman, 2013). It is important to highlight that the observed NO scavenging activity of honey in the present study can prevent the reaction of NO with superoxide ($O_2^{\bullet -}$) to form the much more powerful oxidant peroxynitrite ($ONOO-$) which can be more genotoxic and cause more damage to biomolecules (Pacher et al., 2007). There is also evidence of the potentiating effect of honey on conventional chemotherapeutic agents. For instance, Malaysian Tualang honey was found to promote apoptotic cell death induced by Tamoxifen in MCF-7 and MDA-MB-231 breast cancer cell lines (Yaacob et al., 2013). In addition, the study of Fernandez-Cabezudo et al. (2013) revealed the potential of Manuka honey to reduce Paclitaxel-induced toxicity in mice. Therefore, investigating the combined effect of honey of different floral sources with current chemotherapeutic drugs to increase activity, reduce the required dosage or severity of associated adverse effects would be of considerable interest.

3.7. Phytochemical composition

Preliminary phytochemical screening of the two honey samples revealed the presence of alkaloids, phenols, flavonoids, saponins, steroids, and anthraquinones while steroids were absent (Table 4). Regarding the quantitative phytochemical composition (Table 5), variations were observed among the tested samples such that the TPC of CH (1032.42 µg GAE/g) was found to be significantly greater compared to MH (794.29 µg GAE/g) ($p < 0.05$). A similar pattern was observed for TFC (CH = 80.94 µg RE/g; MH = 69.17 µg RE/g). Similarly, CH displayed higher TC (1352.93 µg CE/g) than MH (621.06 µg CE/g). It is to be noted that these phytochemicals are known to be responsible for the reported bioactivities in the present study (Cowan, 1999; Zhang et al., 2015). For instance, the study of Portokalakis et al. (2016) observed a high correlation between the cytotoxicity of Manuka honey towards MCF-7 cell lines and its phenolic content and antioxidant power. However, in the present study, although only MH displayed anticancer activity against MCF-7 cell line, its phenolic content was lower compared to that of CH. In addition, a negative correlation was observed between TPC, TFC, TC, and the antioxidant activity of honey (R = −1.000). (Table 7).

Table 4
Qualitative phytochemical composition of honey samples.

Phytochemicals	Sample	
	MH	CH
Alkaloids	+	+
Phenols	+	+
Flavonoids	+	+
Saponins	+	+
Steroids	–	–
Anthraquinones	+	+

Note: MH: Mauritian Eucalyptus honey; CH: Commercial honey.

Table 5
Quantitative phytochemical composition of honey samples.

Sample	Total phenolic (µg GAE/g)	Total flavonoid (µg RE/g)	Total tannin (µg CE/g)
MH	794.29 ± 0.52[b]	69.17 ± 1.74[b]	621.06 ± 2.58[b]
CH	1032.42 ± 6.62[a]	80.94 ± 1.49[a]	1352.93 ± 4.76[a]

Note: Values represent mean of triplicate ± standard deviation (n = 3), MH: Mauritian Eucalyptus honey; CH: Commercial honey; Different letter superscript within columns means significantly different ($p < 0.05$).

In fact, the non-correlation between TPC and antioxidant activity observed in the present study was found to be in agreement with the study of Idris et al. (2011), although a number of studies observed significant correlations (Beretta et al., 2005; Chua et al., 2013; Vaghela and Reddy, 2016). This variation is probably due to the qualitative nature of these compounds besides their quantity. It might be that the different compounds in small concentrations act synergistically or specific phenolic and flavonoid compounds with stronger biological activity are present in higher concentrations. Although several studies have demonstrated that raw honey possess higher phenolic and flavonoid content compared to processed honey (Kishore et al., 2011; Muruke, 2014), the opposite was observed in our study. This can be explained by the fact that thermal processing of honey can either decrease or increase its TPC and TFC as found by several studies (Šarić et al., 2013; Turkmen et al., 2006; Wang et al., 2004).

3.8. Physicochemical properties

The physicochemical properties of the tested samples including pH, TSS, colour, and density are shown in Table 6. MH displayed slightly lower pH (3.28) than CH (3.41) although no significant difference was observed statistically ($p > 0.05$). The lower pH of MH compared to CH might be a factor responsible for its greater antibacterial activity as mentioned previously. In addition, the lower pH of MH might be due to the presence of higher amount of specific organic acids which may have contributed to its higher antioxidant activity (Pereira et al., 2009). In fact, a strong positive correlation (R = 1.000) was observed between pH and the extracellular antimelanogenic of honey. On the other hand, pH was negatively correlated (R = −1.000) with NO scavenging and MCF-7 inhibitory activity (Table 7). Compared to other Mauritian honeys, the pH of MH was found to be lower than Wild pepper honey (4.67) and Litchi honey (4.20) (Kinoo et al., 2012). Overall, the pH of the tested honey were found to be close to those previously reported in honey samples around the world (Boussaid et al., 2018; El Sohaimy et al., 2015; Ouchemoukh et al., 2007; Shahnawaz et al., 2013).

Table 6
Physicochemical properties of honey samples.

		MH	CH
pH		3.28 ± 0.05 [a]	3.41 ± 0.11 [a]
Colour	L* a*	81.24 ± 0.38 [a]	75.15 ± 0.42 [b]
	b*	0.92 ± 0.28 [b]	5.68 ± 0.67 [a]
Total soluble solids (°Brix)		33.04 ± 1.72 [b]	49.38 ± 1.07 [a]
Density (g/ml)		79.1 ± 0.98 [a]	80.2 ± 0.56 [a]
		1.55 ± 0.01 [b]	1.71 ± 0.01 [a]

Note: Values represent mean of triplicate ± standard deviation (n = 3); MH: Mauritian Eucalyptus honey; CH: Commercial honey; (−): not detected; Different letter superscript between columns means significantly different ($p < 0.05$).

Table 7
Correlation between TPC, TFC, TC, colour, pH, and the observed pharmacological activities.

Assay	TPC	TFC	TC	L*	a*	b*	pH
NO scavenging activity	−1.000	−1.000	−1.000	1.000	−1.000	−1.000	−1.000
Extracellular antimelanogenic activity	1.000	1.000	1.000	−1.000	1.000	1.000	1.000
MCF-7 inhibitory activity	−1.000	−1.000	−1.000	1.000	−1.000	−1.000	−1.000

Note: Diameter of inhibition zones includes diameter of disc and well (5 mm); ZOI of 5 mm indicates no inhibition; MH: Mauritian Eucalyptus honey; CH: commercial honey.

In addition, the results for TSS (°Brix: CH = 80.2; MH = 79.1) showed no significant differences between the two honey samples ($p > 0.05$). Compared to other Mauritian honeys, the TSS of MH was found to be slightly lower than that of Wild pepper (80.33 °Brix) and Litchi honey (80.50 °Brix) (Kinoo et al., 2012). Overall, the TSS of the honey samples tested in our study were in the range found in literature (75.2–82.17 °Brix) (Idris et al., 2011; Kinoo et al., 2012; Shahnawaz et al., 2013). On the other hand, significant differences were observed in the density of the tested samples such that CH (1.71 g/ml) exhibited significantly higher density than MH (1.55 g/ml) ($p < 0.05$). The density of honey was found to be close to the range (1.056–1.55) reported by (El-Bialee and Sorour, 2011; Kinoo et al., 2012; Manzoor et al., 2013). The slightly higher density observed in CH compared to MH could be due to several reasons mentioned previously for the variations among honey.

Comparison of the colour of the tested samples revealed that MH showed significantly higher L* value, lower a* and b* values (L* = 81.24, a* = 0.92, b* = 33.04) than CH (L* = 75.15, a* = 5.68, b* = 49.38) ($p < 0.05$). Interestingly, strong positive correlation (R = 1.000) was observed between L* and the antioxidant activity of honey, while a* and b* values showed strong negative correlation (R = −1.000) (Table 7). Compared to previous studies, the present findings were in agreement with the study of Kinoo et al. (2012) who found that commercial honeys are darker than raw honey. However, the colour of honey also depends on the botanical sources of nectar as studies of Tunisian and Azerbaijan honey samples showed lower L* values (18.95–51.37) and b* values (0.47–17.67) compared to the honey tested in the present study (Boussaid et al., 2018; Mehryar et al., 2013). Other possible reasons could be the effect of storage or thermal processing leading to non-enzymatic browning reactions including Maillard reaction, formation of intermediates compounds, e.g. brown pigment formation, which may results in increased colour intensity (Turkmen et al., 2006). The colour intensity of honey may also reflect its phenolic content and antioxidant capacity as observed by several studies (Kek et al., 2014; Pontis et al., 2014; Saxena et al., 2010), due to the presence of pigments with antioxidant properties, such as carotenoids and some flavonoids (Meslem et al., 2013). In contrast, in the present study, although the darker honey (CH) displayed higher phenolic content, it exhibited lower antioxidant, extracellular antimelanogenic, and MCF-7 inhibitory activity compared to MH (lighter honey) which can be explained by the importance of qualitative phenolic compounds in addition to its quantity, as mentioned previously.

4. Conclusion

From the present investigation, we found that honey possesses major bioactive phytochemicals and significant pharmacological activities including antibacterial, antioxidant, antimelanogenic, and anticancer activity (against MCF-7 cell). We observed variation among the two honey samples tested such that MH displayed higher antibacterial activity than CH against most tested bacteria. Similarly, higher NO scavenging and MCF-7 inhibitory activity was exhibited by MH although CH showed higher extracellular antimelanogenic activity. Interestingly, the total phenolic, flavonoid, and tannin content was found to be higher in CH. The two honey samples also displayed significant variation in physicochemical properties including colour and density

During the course of this study, we observed certain research gaps which need to be addressed in future studies. One limitation of the current study is the lack of microdilution assay to determine the bacteriostatic or bactericidal effect of honey. In addition, antioxidant assays against other free radicals could be performed to establish the complete antioxidant profile of the samples. The inhibitory properties of honey against other key enzymes involved in the aetiology of non-communicable diseases can also be explored at a higher concentration than the ineffective concentration observed in our study. The anticancer activity of Mauritian honey against MCF-7 cell line indicates its potential activity against other cancer cell lines which need to be validated. Moreover, further work may emphasize the isolation and characterization of bioactive compounds from honey and study the factors responsible for the variations observed among the physicochemical as well as its bioactive properties for the formulation of more potent bio-products. Finally, it is high time to validate the observed pharmacological activities together with toxicological analysis *in vivo* and clinically to obtain the therapeutic dose for the treatment and/or management of both communicable and noncommunicable diseases.

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