UNIVERSIDADE D
COIMBRA

Ana Margarida Monteiro Herdade

Relatório de Estágio e Monografia "Plant-mediated green synthesis of metal-based nanoparticles intended for dermopharmaceutical and cosmetic applications" referente à Unidade Curricular "Estágio", sob orientação do Dr. Paulo Jorge da Silva Monteiro e do Professor Doutor António Henrique Silva Paranhos e apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas

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Setembro de 2020

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Coimbra, 14 de setembro de 2020.

Ana Margarida Monteiro Herdade

(Ana Margarida Monteiro Herdade)

"A Society Grows Great When Old Men Plant Trees
Whose Shade They Know They Shall Never Sit In"
Ricky Gervais

Agradecimentos

"No one who achieves success does so without acknowledging the help of others."
Alfred North Whitehead

À minha avó, a minha maior fã. Obrigada por me ter feito crescer a confiar nas minhas capacidades e a acreditar que "tinha testa de inteligente", por me ter ensinado que o nosso valor está na nossa educação e não no que temos, por me ter mostrado que só com esforço e trabalho árduo se atingem os objetivos, por ser o maior exemplo de que a dedicação ao que fazemos e aos que amamos será sempre recompensada. Sinto muita falta do seu beijo repenicado, mas sei que estará orgulhosamente a bater palmas. A si, dedico este trabalho.

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"E levas em ti guardado
O choro de uma balada
Recordações de um passado
E o bater da velha cabra".

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Relatório de Estágio em Farmácia Comunitária

Farmácia São José

Lista de Abreviaturas

AINE, Anti-inflamatórios não esteroides

ANF, Associação Nacional das Farmácias

CHUC, Centro Hospitalar Universitário de Coimbra

CIMPI, Centro de Informação do Medicamento de Preparação Individualizada

CNP, Código Nacional de Produto

COVID-19: Doença por Coronavírus 2019; do inglês *Coronavirus Disease* 2019

DCI, Denominação Comum Internacional

EEE, Espaço económico europeu

EPI, Equipamento de Proteção Individual

FSJ, Farmácia São José

INFARMED, I.P., Autoridade Nacional do Medicamento e Produtos de Saúde, I.P.

IPO, Instituto Português de Oncologia

MICF, Mestrado Integrado em Ciências Farmacêuticas

MNSR, Medicamento Não Sujeito a Receita Médica

MNSRM-EF, Medicamento Não Sujeito a Receita Médica de Dispensa Exclusiva em Farmácia

OMS, Organização Mundial de Saúde

PVP, Preço de Venda ao Público

SA, Substância Ativa

SABA, Solução Antissética de Base Alcoólica

SNS, Sistema Nacional de Saúde

SWOT, *Stregths, Weaknesses, Opportunities and Threats*

Introdução

A etapa final do Mestrado Integrado em Ciências Farmacêuticas (MICF) corresponde ao estágio em Farmácia Comunitária. Este tem como objetivo preparar o aluno para o mundo profissional, através da sua integração numa equipa em contexto real de trabalho, permitindo colocar em prática todos os conhecimentos adquiridos, mas também ganhar competências que lhe permitam dar resposta perante os desafios diários a que está exposto, tornando-o num verdadeiro farmacêutico.

A farmácia está na primeira linha nos cuidados de saúde pública, sendo o local de eleição do utente para resolver os seus problemas de saúde. Desta forma, o farmacêutico prende-se como o profissional de saúde mais próximo do utente que, além de indicação terapêutica, faz também acompanhamento regular, o que fortalece a sua responsabilidade social. De realçar a importância do plano de estudos do MICF que, graças à diversidade de áreas que abrange, fornece as ferramentas necessárias para que o aluno se torne num especialista do medicamento e um agente de saúde pública.

Serve o presente relatório para fazer um balanço do Estágio em Farmácia Comunitária, realizado na Farmácia São José (FSJ), em Coimbra, sob orientação do Dr. Paulo Monteiro. O período de estágio decorreu entre 3 de fevereiro de 2020 e 7 de agosto de 2020, tendo sido interrompido de 18 de março de 2020 a 4 de maio de 2020, devido à Declaração do Estado de Emergência [1]. O relatório será feito na forma de análise *SWOT*, acrónimo de *Strenghts, Weakness, Opportunities e Threats*, que inclui uma análise quer a nível interno, avaliando os pontos fortes e fracos, quer a nível externo, avaliando oportunidades e ameaças com que me deparei durante este período.

Farmácia São José

A Farmácia São José foi aberta ao público em janeiro de 1950, nos Arcos do Jardim, em Coimbra. Passados 7 anos, a farmácia trespassou para a Dra. Maria Prazeres Monteiro que, em 1984 alterou a localização da farmácia para a Avenida Calouste Glubenkian, em Celas. O Dr. Paulo Monteiro, filho da proprietária, tornou-se diretor técnico em 1997 mantendo-se, desde então, na gerência da farmácia.

Dado o enorme crescimento que a FSJ teve ao longo dos anos, em 2004 foram feitas obras, nomeadamente instalação do *robot* e aumento da área, tendo sido renovado em 2019, com o intuito de aumentar a capacidade de armazenamento e aprovisionar o stock de medicamentos. Neste momento estão a começar novas obras, que visão a renovar totalmente o *layout* da farmácia.

1. *Strengths* - Pontos Fortes

1.1 Localização, Horário de Funcionamento e Utentes

Um dos pontos mais fortes da FSJ é exatamente a sua localização. A sua proximidade ao Centro Hospitalar e Universitário de Coimbra (CHUC), ao Instituto Português de Oncologia (IPO), à Liga Portuguesa Contra o Cancro, entre outros centros clínicos e também comércio variado, tornam a FSJ num local de eleição.

A sua localização e o fácil acesso (a pé, por transportes públicos ou por transporte privado) leva a que a população abrangida seja muito heterogénea, o que se traduz em diferentes faixas etárias e extratos socioeconómicos. Desta forma, pude contactar com variadíssimas patologias, prescrições, manipulados, mas também aprender imenso sobre o serviço de quase "psicólogo" e ombro amigo que o farmacêutico presta, principalmente à população mais idosa, o que me fez crescer muito enquanto profissional e pessoa.

O horário de funcionamento alargado (de segunda a sábado, das 8h30 às 21h) permite que todos os utentes, desde os mais livres aos que têm horários mais ocupados, possam deslocar-se à farmácia sempre que precisam, o que contribui para a já mencionada heterogeneidade da população abrangida pela FSJ. Para além disso, nos dias de serviço permanente, está aberta 24h, garantindo assim o acesso a medicação de urgência. Este horário foi também uma vantagem para mim, porque permitiu ter maior flexibilidade nos horários do estágio e ter a possibilidade de estagiar durante momentos distintos do dia, em que o tipo de utente e o ritmo de trabalho são totalmente diferentes.

Adicionalmente, devido ao serviço exímio que sempre prestou, a maioria dos clientes têm a FSJ como preferência desde o começo, que se reflete quando mencionam "o tempo dos Arcos do Jardim" ou "o Dr. Paulo em pequenino". A história já longínqua da farmácia e o serviço de excelência colocaram-na numa posição de referência em Coimbra, o que se reflete na abrangente população que a ela recorre.

Como já referido, os utentes que têm a FSJ como farmácia de eleição são muito diferentes, desde jovens a idosos, o que se reflete também no atendimento. A diferente faixa etária e capacidade monetária criam a oportunidade de lidar com as mais variadas situações, o que contribuiu para o meu crescimento em todas as áreas da farmácia (medicação crónica e dúvidas associadas, indicação terapêutica para situações mais pontuais, acompanhamento farmacêutico, aconselhamento cosmético, suplementação, etc.).

1.2 Organização e Gestão do espaço da Farmácia

Na FSJ, os medicamentos não sujeitos a receita médica (MNSRM) encontram-se visíveis, mas inacessíveis ao público, contrariamente aos produtos de venda livre (dermocosmética, podologia, ortopedia, perfumaria, entre outros), a que os utentes têm acesso. Na sua maioria, os produtos expostos estão separados por sintomatologia, o que contribui para um atendimento mais rápido. A disposição é elaborada tendo em conta os conceitos de *Marketing*, utilizando as zonas quentes para produtos sazonais e prioritários. Desta forma, a organização e gestão do espaço da farmácia permitiram-me compreender a importância das técnicas de *Marketing* para dinamizar as vendas, o que se traduz numa maior rotatividade de produtos e maior rentabilidade para a farmácia.

1.3 Satisfação do Cliente

Durante o estágio, um dos principais ensinamentos que me foi passado é que satisfazer a necessidade do cliente é sempre a nossa prioridade. Desde o pedido mais singular em que é preciso "correr mundos e fundos" para conseguir, até ao pedido mais trivial de uma simples encomenda instantânea a um armazenista, ou até o simples ato de ouvir o utente e prestar-lhe apoio, todos os pedidos são atendidos. Não há missões impossíveis e é nítido que os utentes procuram a FSJ exatamente pela "fama" de atender a todo e qualquer pedido, o que leva à sua satisfação e, consequentemente, fidelização.

Para gerir os pedidos dos utentes, a FSJ criou um sistema informático de reservas, disponível em todos os computadores sobre a forma de um ficheiro *Excel®* no *Google Docs®*. Neste documento são registados todos os dados importantes no momento do pedido (nome e contacto do utente, nome, Código Nacional de Produto - CNP e quantidade do produto, fornecedor, operador que fez o pedido, data e hora e indicação se o produto ficou pago ou em dívida); aquando da receção do produto encomendado, o operador consulta novamente o documento para acusar a sua receção e indicar o local de armazenamento (a FSJ dispõe de locais próprios no *Back Office*); finalmente, ao entregar o produto ao utente, o operador volta a usar o documento para comunicar a sua entrega. Desta forma, os pedidos de produtos e o respetivo stock estão sempre controlados e qualquer operador consegue facilmente seguir o seu fluxo.

1.4 Equipa

Uma boa gestão dos recursos humanos é fundamental para o bom funcionamento e sucesso da farmácia. A numerosa e multidisciplinar equipa da FSJ faz com que cada funcionário tenha um papel bem definido, o que contribui para uma boa organização das tarefas e uma boa

gestão. Os funcionários têm papeis variados, entre eles: gestão comercial, trabalho de *Back Office* associado a encomendas e relações com armazenistas, atendimento geral e funções específicas como conferir o receituário, preparação individualizada da medicação, preparar medicamentos manipulados ou ter destaque em determinada área, devido aos conhecimentos aprofundados - como é o caso da cosmética. Devido à multidisciplinaridade da equipa, tive a oportunidade de aprender todas as valências da farmácia comunitária, o que permitiu não só pôr em prática e consolidar os conhecimentos adquiridos no MICF, como expandir as minhas capacidades e aptidões, nomeadamente a nível da gestão comercial, relações com armazenistas e fornecedores e nos serviços prestados pela farmácia.

1.5 Cosmética

A FSJ tem uma vasta oferta de produtos de cosmética, que inclui todas as marcas de referência no mercado e a grande maioria das gamas dos mesmos. Mais importante ainda, há uma funcionária com destaque para a cosmética que, dados os seus conhecimentos aprofundados na área, consegue dar o melhor aconselhamento ao utente, fidelizando-o. Além disso, as campanhas e ações com promotoras das marcas acontecem com muita frequência, o que impulsiona as vendas. Claramente, isto é uma enorme vantagem, que coloca a FSJ num lugar de destaque em relação à concorrência. A cosmética desempenha, cada vez mais, um papel fulcral nas vendas da farmácia e os utentes têm uma crescente preocupação com os cuidados da pele, pelo que ter uma vasta oferta e ter capacidade para prestar o aconselhamento adequado é essencial. Desta forma, considero que isto foi uma mais valia para o meu estágio, tendo permitido não só alargar os conhecimentos obtidos na cadeira de Dermofarmácia e Cosmética, como também contactar e conhecer aprofundadamente toda a oferta de cosméticos.

1.6 Medicamentos Manipulados

Segundo a Portaria nº594/2004, de 2 de junho de 2004, sobre as Boas Práticas para a Preparação de Medicamentos Manipulados em Farmácia Comunitária e Hospitalar, um manipulado é "qualquer fórmula magistral ou preparado oficinal, preparado e dispensado sob a responsabilidade de um farmacêutico". A preparação de medicamentos manipulados é feita por farmacêuticos, o que reforça a sua importância na saúde da comunidade, num laboratório com todas as estruturas e equipamentos necessários.

Inicialmente faz-se a validação da prescrição do medicamento manipulado, seguida do preenchimento da ficha de preparação através de um *software* que a FSJ dispõe para gerir toda a informação relativa à preparação de manipulados, o *Soft Galeno*®. Este permite fazer a gestão

dos *stocks* das matérias-primas, consultar fichas de preparações anteriormente realizadas ou criar uma nova ficha. Após esta fase inicial, faz-se a preparação do manipulado e, finalmente, procede-se ao controlo de qualidade, embalamento, rotulagem e cálculo do preço do respetivo medicamento. A ficha de preparação é então datada, carimbada e assinada pelo farmacêutico responsável e arquivada pelo número de lote atribuído.

Na FSJ, a maioria dos medicamentos manipulados são formulações para uso tópico, para uso veterinário ou para a população pediátrica, visto que são situações específicas para as quais não existem alternativas à venda, seja por necessitar de diferente dose, forma farmacêutica ou composição. Quando há alguma dúvida na preparação, é enviado um pedido de informação ao Centro de Informação do Medicamento de Preparação Individualizada (CIMPI), que avalia o pedido e fornece todos os esclarecimentos necessários. Praticamente todos os dias chegavam pedidos de manipulados, o que foi uma enorme vantagem educacional, tendo-me permitido fazer várias formulações diferentes e, assim, praticar os conhecimentos adquiridos nas unidades de Farmácia Galénica e Tecnologias Farmacêuticas. Um dos medicamentos manipulados em que tive oportunidade de participar foi uma Suspensão Oral de Piridostigmina 20 mg/mL, para uso pediátrico, como anticonvulsivante (uso *off-label*). Em anexo encontra-se a ficha de preparação, a receita prescrita pelo médico e imagens da matéria-prima, da preparação e do manipulado embalado e rotulado.

1.7 Serviços Prestados

A farmácia não só é o local onde são dispensados medicamentos e produtos de saúde, mas também onde são prestados os mais variados serviços, o que enfatiza o seu reconhecimento por parte dos utentes como um estabelecimento de saúde e bem-estar. Um dos serviços farmacêuticos mais procurados é a medição de parâmetros bioquímicos e fisiológicos (peso, glicémia, triglicéridos, colesterol, pressão arterial). Estas medições permitem ao utente, de uma forma acessível e fácil, monitorizar estes parâmetros com frequência, além de ter o resultado interpretado por um profissional de saúde; além disso, se o utente for fidelizado, o acompanhamento a longo prazo permite ao farmacêutico uma melhor análise dos resultados e aconselhamento ao utente. Devido à pandemia da Doença por Coronavírus 2019 (COVID-19), este tipo de serviço foi suspenso, tendo sido reposto, com todos os cuidados devidos, apenas no final do meu estágio, pelo que não tive oportunidade de o praticar com frequência. Outro serviço de destaque é a administração de medicamentos injetáveis, efetuada por farmacêuticos com formação específica, reconhecida pela Ordem dos Farmacêuticos. Além destes serviços, a FSJ tem ainda ao dispor dos utentes consultas de nutrição, realizadas semanalmente por uma nutricionista, destinadas tanto a

utentes com regimes alimentares especiais (por exemplo para diabéticos e hipertensos) como a utentes que queiram alterar os seus hábitos alimentares e ajustá-los às suas necessidades. A FSJ dispõe de diversos suplementos alimentares que poderão ser aconselhados pela profissional, de forma a complementar a sua dieta, o que leva à satisfação e fidelização dos utentes. Todos estes serviços farmacêuticos, de forma a conferir maior privacidade e conforto ao utente, são realizados num gabinete de atendimento personalizado. Devido à época pandémica em que vivemos, o serviço de entregas ao domicílio foi extensamente procurado, pelo que assumiu um papel central no meu estágio. A FSJ sempre teve ao dispor dos utentes as entregas ao domicílio com um custo adicional, mas durante a pandemia este serviço aumentou exponencialmente, tendo-se tornado gratuito e tendo sido complementado com a possibilidade de encomendas por via telefónica ou por e-mail. Estas alterações permitiram zelar pela saúde dos utentes, que não precisariam assim de sair das suas casas para adquirir a medicação habitual e outros produtos de saúde necessários, zelando também pela saúde dos colaboradores, mantendo a farmácia com o mínimo de fluxo possível. Durante o estágio este foi o serviço com maior destaque, que se demonstrou essencial para o cumprimento do distanciamento social e para a manutenção do bem-estar dos utentes. Considero que todos os serviços farmacêuticos prestados são cruciais para destacar a FSJ das restantes e para promover um contacto mais próximo e uma maior relação de confiança com os utentes, levando à sua fidelização. Além disso, estes serviços contribuem diretamente para uma melhor qualidade de vida da população, o que realça o papel da farmácia comunitária na sociedade.

2. *Weaknesses* - Pontos Fracos

2.1 Nome Comercial do Medicamento

Para mim esta foi uma das maiores dificuldades durante o estágio. Durante o MICF aprendemos tudo o que precisamos sobre as substâncias ativas (SA) que compõe o medicamento; no entanto, temos pouco conhecimento sobre os nomes comerciais dos medicamentos com essas mesmas SA. A relação entre o nome comercial e a denominação comum internacional (DCI) foi uma grande dificuldade durante o atendimento, dado que muitos utentes pedem a medicação pelo nome de marca, o que me levava a ter de recorrer ao SIFARMA2000® ou a pedir ajuda a outro elemento da equipa. Algo que acontecia recorrentemente era os utentes pedirem informações de um medicamento pelo nome comercial ou simplesmente pedirem o medicamento, mas dizerem o nome mal, o que fazia com que eu demorasse muito tempo para o decifrar, tal como à SA em questão. Esta demora no atendimento era muitas vezes vista como falta de conhecimento por parte do utente, o que criava insegurança no meu aconselhamento enquanto estagiária.

2.2 Organismos de Comparticipação

Além do Sistema Nacional de Saúde (SNS), existem outros subsistemas de saúde para a comparticipação de medicamentos e produtos de saúde, que resultam de acordos entre a Associação Nacional das Farmácias (ANF) e diversas entidades (seguradoras, sindicatos, entre outros). Todos os organismos de comparticipação estão listados no SIFARMA2000® mas, devido ao desconhecimento que os estagiários têm em relação aos mesmos e ao respetivo código, este processo torna o atendimento mais moroso; no entanto, é algo que com a prática se vai dissipando.

2.3 Homeopatia e Medicamentos de Uso Veterinário

Estas são duas áreas em que me deparei com grandes dificuldades. Apesar da falta de evidência científica que comprove a eficácia dos produtos homeopáticos, estes são muitas vezes solicitados pelos utentes e, dado que a formação sobre os mesmos no MICF é praticamente nula, tive muita dificuldade em aconselhar. Para além destes, também me senti muito insegura no aconselhamento sobre medicamentos de uso veterinário, visto que o ensino que temos durante o MICF nesta área é muito breve e não nos prepara para saber aconselhar e tirar dúvidas sobre os mesmos.

3. *Opportunities* - Oportunidades

3.1 Medicamentos Não Sujeitos a Receita Médica de Dispensa Exclusiva em Farmácia

Os Medicamentos Não Sujeitos a Receita Médica de Dispensa Exclusiva em Farmácia (MNSRM-EF) podem ser dispensados sem prescrição médica, mas a sua dispensa está vedada à farmácia dado que, pelas suas características, carecem de aconselhamento farmacêutico. Esta subcategoria de medicamentos valoriza a farmácia e a intervenção do farmacêutico no aconselhamento e dispensa dos mesmos, o que se traduz também numa maior segurança para o utente, através do uso eficaz, seguro e racional dos medicamentos.

3.2 Formações

'Tell me and I forget. Teach me and I remember. Involve me and I learn.'

- Benjamin Franklin

O MICF fornece as ferramentas necessárias para a formação de um farmacêutico, mas apenas com a contínua curiosidade e aprendizagem é possível evoluir e ser-se um bom profissional. Na FSJ, a formação dos seus trabalhadores e dos estudantes é parte integrante

do dia-a-dia. As formações, tanto internas como externas à farmácia, são essenciais para correlacionar os conhecimentos teóricos com os produtos disponíveis no mercado, facilitando assim o momento de aconselhamento farmacêutico.

Antes do começo da pandemia, tive ainda a possibilidade de assistir a uma formação fora da farmácia patrocinada pela Arkopharma e, no início da pandemia, assisti à formação da Plural cujo intuito era elucidar estagiários e trabalhadores sobre a COVID-19. Durante o pico da pandemia as visitas presenciais foram suspensas, pelo que só recentemente voltei a ter a possibilidade de participar em formações, desta vez feitas por delegados de informação médica na farmácia (Viv Oral, Fresenius, *Àvene®*, entre outros).

3.3 COVID-19

A pandemia da covid-19, apesar de ter sido vista como uma ameaça por parte de muitos colegas, tornou-se para mim numa grande oportunidade de aprendizagem e de pôr em prática os deveres e valores desta profissão. O farmacêutico, além de especialista do medicamento, é também um agente de saúde pública, pelo que recai sobre ele o dever de promover a literacia na área da saúde, de forma a promover a saúde dos utentes e da sociedade no geral.

Graças à enorme afluência à farmácia no início da pandemia para comprar Equipamentos de Proteção Individual (EPIs) e medicação para situações agudas (nomeadamente analgésicos, anti-inflamatórios, entre outros), a pandemia permitiu-me contactar diretamente com fornecedores e aprender de forma aprofundada o processo de compra. Devido à falta de funcionários, acabei mesmo por me tornar uma das responsáveis por todos os EPIs da farmácia, o que me permitiu efetuar de forma autónoma todo o seu processo de compra: procura dos mesmo e discussão com os fornecedores para obter informações dos mesmos e condições de compra e de envio, escolha do fornecedor e dos produtos, compra e pagamento, receção dos produtos e dar entrada dos mesmos no sistema (o que inclui criar margens, preços e etiquetar os produtos). Devido à enorme procura de EPIs, estes rapidamente esgotaram, o que me obrigou a aprender a procurar novos fornecedores, a arranjar alternativas e impulsionou a minha capacidade organizacional, levando-me a criar uma lista de contactos com todas as especificações de cada fornecedor, que era atualizada diariamente, de forma a agilizar todo o processo de compra e a compilar as informações relevantes. A pandemia permitiu-me também pôr em prática tudo o que aprendi sobre regulamentação na saúde e sobre veracidade de informação. Sendo a COVID-19 uma novidade, tive de ler regulamentos e decretos, estar sempre a par das declarações políticas e das entidades de saúde e ler imensos artigos sobre o vírus (principalmente sobre a prevenção

e o tratamento) e sobre os EPIs. A pandemia permitiu-me também fazer um manipulado que normalmente não seria necessário, neste caso a Solução Antissética de Base Alcoólica (SABA), preconizado pela Organização Mundial de Saúde (OMS).

Esta situação também me fez ter uma melhor noção do papel da farmácia na comunidade: com a falta de consultas médicas a população recorreu aos farmacêuticos para tirar dúvidas relativas a medicação e, principalmente, relativas à informação que diariamente mudava sobre o vírus, para pedir aconselhamento terapêutico, e mesmo para lidar com o stress e solidão. A farmácia teve o seu papel enaltecido, demonstrando uma vez mais que a sua grande missão é servir a comunidade, fazendo as adaptações necessárias a cada altura. Isto notou-se, por exemplo, pelo serviço de encomendas e entrega ao domicílio, referido anteriormente. Considero que a pandemia demonstrou uma vez mais que o farmacêutico é um agente de saúde pública que está capacitado para responder às necessidades da população e que, mesmo nas horas mais adversas, dedica a sua vida a cuidar da comunidade.

4. *Threats* - Ameaças

4.1 Medicamentos Esgotados e Produtos Rateados

Portugal está inserido no espaço económico europeu (EEE), onde o mercado é livre e permite a transferência de produtos, incluindo medicamentos. A baixa densidade populacional do nosso país e o facto dos preços praticados sob os medicamentos serem baixos, em comparação com os outros países do EEE, faz com que uma boa percentagem dos medicamentos comercializados em Portugal sofra exportação paralela. Este fenómeno leva a que as farmácias se deparem regularmente com medicamentos esgotados, sendo que muitos deles representam medicação crónica, como por exemplo o Victan®, para o qual não há alternativa terapêutica. No entanto, a maioria dos medicamentos dispõe de alternativas terapêuticas - os genéricos, o que ameniza esta situação. Infelizmente, muitos utentes continuam reticentes aos medicamentos genéricos e recusam-se a tomá-los em detrimento do de marca, chegando mesmo a preferir interromper a terapêutica, o que compromete o seu estado de saúde e a qualidade de vida. Recorrentemente os utentes demonstram-se muito frustrados com a falta do medicamento e, dado que não compreendem a razão pela qual este se encontra esgotado, culpabilizam a farmácia. Desta forma, os medicamentos esgotados representam uma ameaça para o normal funcionamento da farmácia, mas também para a saúde pública. Realço ainda que a falta de conhecimento sobre os medicamentos genéricos é uma ameaça enorme para as farmácias e para a saúde pública, pelo que se deveriam investir em ações para promover a literacia em saúde que abordem este tema.

Há ainda medicamentos rateados que, consoante o volume de compras e a fidelização da farmácia na empresa de distribuição, são cedidos em quantidades específicas. Esta racionalização promove a desigualdade no acesso aos medicamentos por parte da população, pelo que o principal prejudicado é o doente, cuja saúde e bem-estar são a prioridade do farmacêutico. Desta forma, considero que a Autoridade Nacional do Medicamento e Produtos de Saúde, I.P. (INFARMED, I.P.) deveria arranjar uma forma de contornar esta situação, promovendo uma melhor distribuição dos produtos tanto para as distribuidoras como para as farmácias, com o objetivo de garantir a igualdade de acesso ao medicamento pela população.

4.2 Alterações no PVP

O INFARMED, I.P. altera, com bastante frequência, o Preço de Venda ao Público (PVP) de alguns medicamentos. A ficha do produto é atualizada imediatamente e apresenta apenas os PVP autorizados pelo INFARMED, I.P. e, a partir desse momento, o medicamento só poderá ser vendido a um desses preços, independentemente do que consta na cartonagem. Desta forma, por vezes acontece um medicamento ser vendido a um preço diferente do que consta na cartonagem, o que causa situações constrangedoras durante o atendimento. A maioria dos utentes desconhece este procedimento e associa esta decisão à farmácia em si, o que põe em causa a credibilidade do farmacêutico.

4.3 Desinformação

Atualmente vivemos na era das tecnologias, em que qualquer informação está à distância de um clique. Infelizmente, esta informação nem sempre é fidedigna, mas não está ao alcance de todos saber diferenciar informação relevante e factual de informação enganosa. Hoje em dia é comum a internet ser o primeiro meio em que procuramos respostas, e isso é bem visível na saúde. É cada vez mais usual ver os utentes recorrerem a motores de busca para procurar soluções rápidas, baratas e eficazes e dirigem-se à farmácia com ideias pré-definidas do que pretendem. Estas soluções, muito frequentemente, não são as corretas para o problema que levou o utente a dirigir-se à farmácia, mas não é fácil fazer com que este compreenda que a informação encontrada online pode não ser fidedigna e que deve seguir o conselho do seu médico e/ou farmacêutico e não o que leu *on-line*. Esta desinformação foi notória durante a pandemia, por exemplo relativamente às notícias de produtos vindos da China ou à notícia sobre a utilização de anti-inflamatórios não esteroides (AINEs), entre outros.

Enquanto agentes de saúde pública considero que temos não só a obrigação de trabalhar de forma a diminuir a desinformação na sociedade, aumentando a literacia em saúde,

mas também de construir fortes relações de confiança com os nossos utentes, para que futuramente sejamos nós o primeiro motor de busca.

4.4 Concorrência

A concorrência entre farmácias faz com que estas se tentem diferenciar, seja pelo atendimento, pelo preço ou pelos serviços prestados, com o objetivo de fidelizar o maior número de utentes. No entanto, mais do que a competição entre farmácias, a competição com outros espaços de saúde e bem-estar em grandes superfícies comerciais é cada vez mais sentida e representa uma ameaça direta para as farmácias, dado que estes espaços oferecem uma grande oferta de produtos a preços mais apelativos, devido ao seu elevado volume de compras. Para contornar esta situação, o *Marketing* é uma ferramenta chave, permitindo oferecer uma grande diversidade de produtos, mantendo uma boa rentabilidade para a farmácia. Para além disso, as farmácias devem distinguir-se não só como espaço comercial, mas também como espaço de saúde, seja pelos serviços prestados como pelo atendimento personalizado e de excelência, realizado por profissionais de saúde devidamente habilitados e com um nível de conhecimento que lhes permite melhorar a qualidade de vida dos utentes, fidelizando-os.

Considerações Finais

Finalizado este percurso, posso afirmar que o estágio em Farmácia Comunitária, obrigatório para finalizar o MICF, é absolutamente essencial e corresponde ao culminar de quatro anos e meio de aprendizagem. Considero que a duração é a ideal, porque me permitiu progredir continuamente conforme o que fui aprendendo, dando-me segurança e confiança para desempenhar as tarefas atribuídas. O MICF preparou-nos muito bem, dando-nos todos os conhecimentos teóricos e as capacidades práticas necessárias para, no contexto real do mercado de trabalho, passar da teoria à prática e conjugar todos esses ensinamentos. Além de todas as áreas em que tive oportunidade de aprofundar os meus conhecimentos, tais como a Dermocosmética e os Medicamentos para Uso Veterinário, adquiri *soft skills* que serão uma mais valia para qualquer que seja o futuro que se avizinha. Cresci intelectual e profissionalmente, mas cresci também muito pessoalmente, tendo aprendido imenso sobre companheirismo, trabalho de equipa e o que significa ser um líder de respeito e que dá o exemplo. Durante o meu estágio percebi também o quão nobre é a profissão farmacêutica e o lugar de destaque que realmente temos, não só por sermos importantes agentes de saúde pública, mas também pelo nosso papel como amigos, ouvintes e companheiros.

Concluindo, retiro um balanço extremamente positivo do meu estágio na Farmácia São José, o qual se deve a toda a equipa e à dedicação e paixão que colocam diariamente na sua profissão. O objetivo do meu estágio foi atingido: saio da farmácia muito mais rica, pessoal e profissionalmente, com confiança nas minhas capacidades e com uma enorme vontade de partir para o mercado de trabalho e pôr em prática todos os conhecimentos adquiridos, visando sempre glorificar a profissão farmacêutica.

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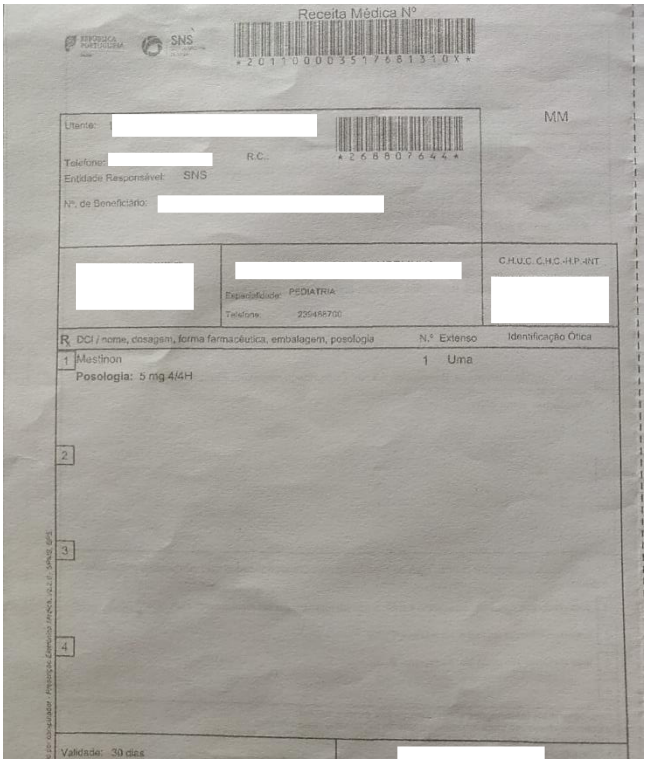
[5] Resumo das Características do Medicamento - Elocom® 1 mg/g pomada. [Acedido: 6 de setembro de 2020] Disponível na Internet: https://extranet.infarmed.pt/INFOMED-fo/pesquisa-avancada.xhtml.

[6] Frezyderm - Anticort® creme [Acedido a 6 de setembro de 2020] Disponível na Internet: https://www.frezyderm.com/specialized-care/alternative-treatment/anticort-cream/?UserAction =True.

Anexos

Anexo I - Ficha de Preparação de um Medicamento Manipulado - Suspensão Oral de Piridostigmina 20 mg/mL



Receita Médica Nº

Utente:

MM

Telefone:

R.C.

Entidade Responsável: SNS

Nº. de Beneficiário:

C.H.U.C.-C.H.C.-H.P.-INT

Especialidade: PEDIATRIA

Telefone: 239488700

R DCI / nome, dosagem, forma farmacêutica, embalagem, posologia | Nº Extenso | Identificação Ótica

1 Mestinon | 1 Uma

Posologia: 5 mg 4/4H

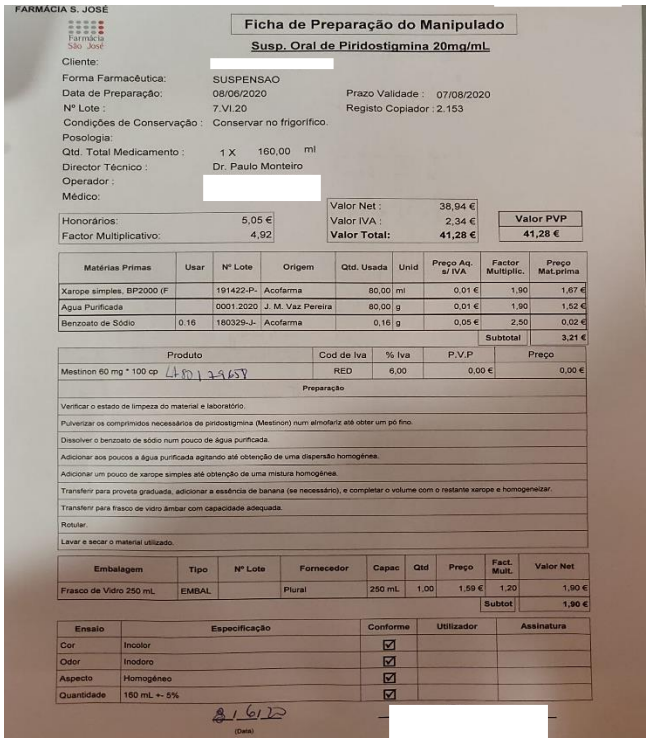
2

3

4

Validade: 30 dias

Fig. A Receita do medicamento manipulado.



FARMÁCIA S. JOSÉ

Farmácia São José

Ficha de Preparação do Manipulado

Susp. Oral de Piridostigmina 20mg/mL

Cliente:

Forma Farmacêutica: SUSPENSAO

Data de Preparação: 08/06/2020 Prazo Validade : 07/08/2020

Nº Lote : 7.VI.20 Registo Copiador : 2.153

Condições de Conservação : Conservar no frigorífico.

Posologia:

Qtd. Total Medicamento : 1 X 160,00 ml

Director Técnico : Dr. Paulo Monteiro

Operador :

Médico:

Honorários:	5,05 €
Factor Multiplicativo:	4,92

Valor Net :	38,94 €
Valor IVA :	2,34 €
Valor Total:	**41,28 €**

Valor PVP 41,28 €

Matérias Primas	Usar	Nº Lote	Origem	Qtd. Usada	Unid	Preço Aq. s/ IVA	Factor Multiplic.	Preço Mat.prima
Xarope simples, BP2000 (F		191422-P-	Acofarma	80,00	ml	0,01 €	1,90	1,67 €
Agua Purificada		0001.2020	J. M. Vaz Pereira	80,00	g	0,01 €	1,90	1,52 €
Benzoato de Sódio	0.16	180329-J-	Acofarma	0,16	g	0,05 €	2,50	0,02 €
							Subtotal	3,21 €

Produto	Cod de Iva	% Iva	P.V.P	Preço
Mestinon 60 mg * 100 cp 4801 [illegible]	RED	6,00	0,00 €	0,00 €

Preparação

Verificar o estado de limpeza do material e laboratório.

Pulverizar os comprimidos necessários de piridostigmina (Mestinon) num almofariz até obter um pó fino.

Dissolver o benzoato de sódio num pouco de água purificada.

Adicionar aos poucos a água purificada agitando até obtenção de uma dispersão homogénea.

Adicionar um pouco de xarope simples até obtenção de uma mistura homogénea.

Transferir para proveta graduada, adicionar a essência de banana (se necessário), e completar o volume com o restante xarope e homogeneizar.

Transferir para frasco de vidro âmbar com capacidade adequada.

Rotular.

Lavar e secar o material utilizado.

Embalagem	Tipo	Nº Lote	Fornecedor	Capac	Qtd	Preço	Fact. Mult.	Valor Net
Frasco de Vidro 250 mL	EMBAL		Plural	250 mL	1.00	1,59 €	1,20	1,90 €
							Subtot	1,90 €

Ensaio	Especificação	Conforme	Utilizador	Assinatura
Cor	Incolor	☑		
Odor	Inodoro	☑		
Aspecto	Homogéneo	☑		
Quantidade	160 mL +- 5%	☑		

8/6/20 (Data)

Fig. B Ficha de preparação do medicamento manipulado.

Fig. C Matéria prima para a preparação do manipulado.

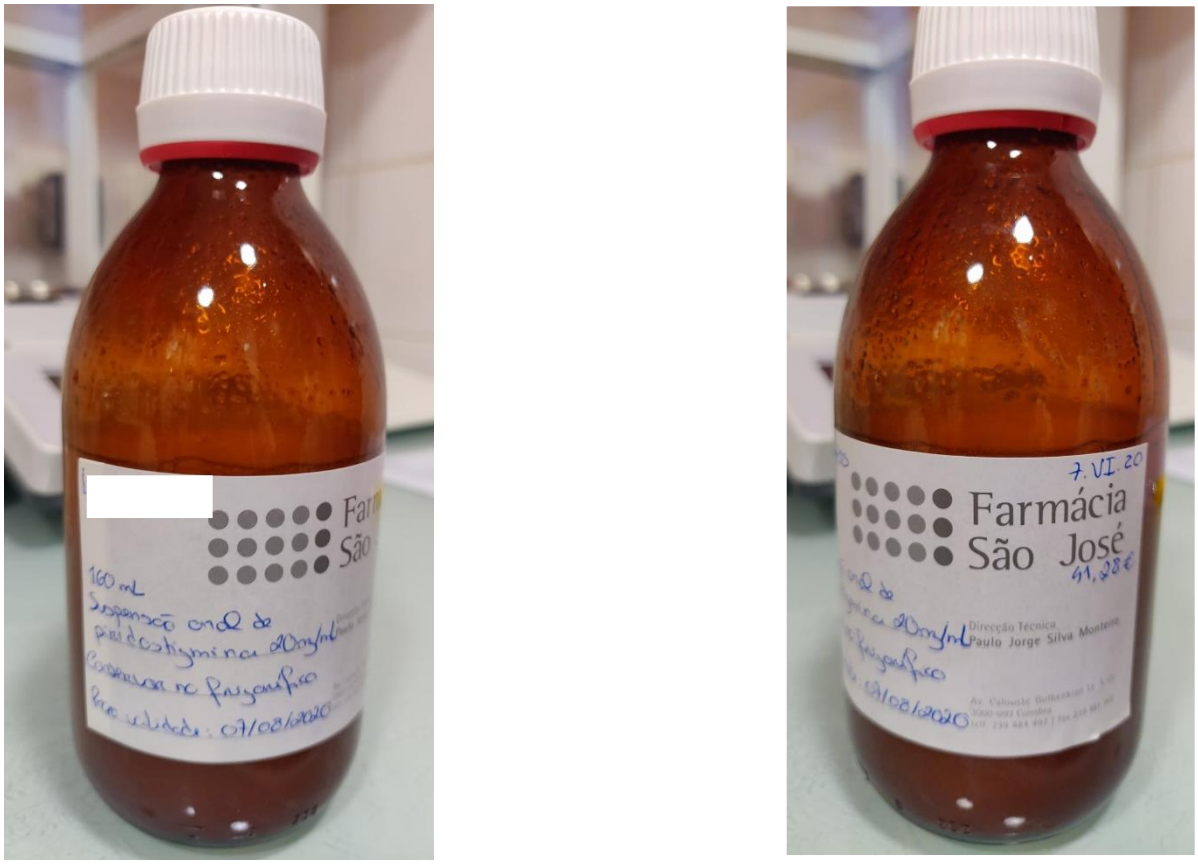


Fig. D Medicamento manipulado, devidamente embalado e rotulado.

Anexo II - Casos Práticos de Aconselhamento Farmacêutico

Caso I

Um senhor, na casa dos 30 anos, dirige-se à Farmácia São José e pede uma pomada para picadas de insetos. De forma a compreender melhor o tipo de picadas e a gravidade, perguntei ao senhor qual o nível de comichão que sentia, se a zona da picada estava dura ou se tinha libertado algum líquido, qual a área que cobriam e se saberia que tipo de inseto o tinha mordido. O senhor mostrou-me as suas costas e tronco, onde pude verificar que existiam inúmeras picadas, extremamente reativas (altas e muito vermelhas), com uma área muito grande que se alastrava de umas para outras. O senhor referiu ainda que as picadas estavam assim pelo corpo inteiro, que libertaram algum líquido e que lhe davam tanta comichão que não conseguia dormir. Tendo em conta todas estas informações, aconselhei ao senhor um gel tópico com dimetindeno (antagonista dos recetores H1 da histamina) - neste caso em particular aconselhei Vittopic®, dado que era o gel que estava no linear na altura, a que deveríamos dar preferência no ato de venda- para aplicar uma camada fina nas zonas das picadas 2 a 4 vezes por dia. Dada a extensão e a reatividade das picadas aconselhei ainda Cetirizina Nargoran® 10mg (antagonista potente e seletivo dos recetores H1 periféricos) para tomar 1 vez por dia, à noite porque, dado que pode provocar alguma sonolência, seria uma ajuda para o senhor conseguir dormir descansado.

Caso II

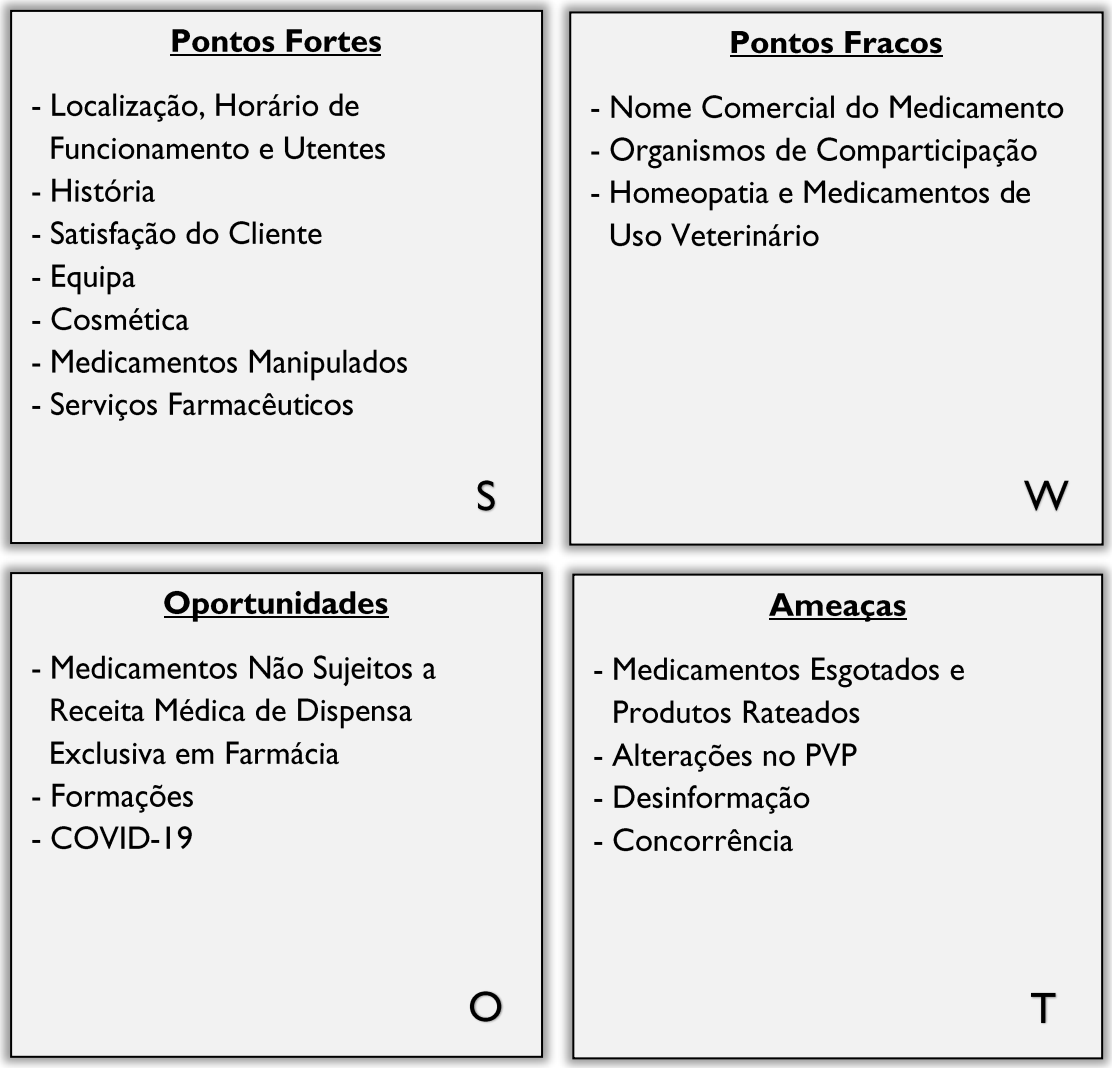
Uma menina nova, por acaso caloira da FFUC, apareceu na farmácia com a queixa de que tinha as mãos com feridas. Para compreender melhor a situação, pedi que me mostrasse as lesões. Verifiquei que efetivamente havia uma lesão na palma da mão, mas também num dos dedos, junto à unha. As lesões eram características de eczema, dado que apresentavam eritema, edema e alguma descamação. Para além disso, a utente queixou-se de prurido intenso e, devido ao ato de coçar, acabou por exacerbar as lesões. De forma a compreender o tipo de eczema, era importante esclarecer o que o provocou. Questionei a utente sobre o aparecimento das lesões, a qual me confessou que estas lesões reapareceram durante a pandemia, mas que já tinha tido no passado. O uso recorrente de soluções antissépticas terá despoletado o eczema, o que me levou a perceber que se trataria de um eczema de contacto, provocado por agentes irritantes. A utente referiu ainda que na altura tinha consultado um dermatologista, que prescreveu Elocom® 1 mg/g pomada (cujo princípio ativo é a mometasona) [5]. Questionei então se o Elocom® tinha funcionado e qual a posologia que tinha feito. Efetivamente, o Elocom® fez o efeito pretendido na altura, mas a utente parou de colocar de

um dia para o outro, sem ter feito o desmame necessário dos corticosteroides. Esta paragem abrupta no tratamento, juntamente com o elevado uso de soluções antissséticas de base alcoólica diretamente na pele terá levado ao efeito *rebound* (ou seja, como o desmame dos corticosteroides não foi feito, o eczema de contacto voltou a aparecer). Como o Elocom® já tinha sido anteriormente prescrito e tinha feito o efeito desejado, e dado que o reaparecimento foi devido à falta de desmame, perguntei à menina se ainda tinha receita médica para o Elocom®. Como tinha receita, e dado que o eczema já estava demasiado evoluído para não aplicar um corticosteroide, aconselhei então novamente o Elocom®. Como terapia complementar, aconselhei também anticort®, um produto à base de compostos naturais e, apesar de não ter corticoides, tem uma ação análoga, ou seja, uma ação *corticoide-like*. Este produto foi uma novidade que conheci e sobre o qual aprendi durante o estágio, e que achei muito interessante tanto como alternativa como para complemento aos corticosteroides tópicos. A posologia que aconselhei à menina para o Elocom® foi:

- durante os primeiros 5 dias aplicar uma camada fina 1 vez por dia, á noite (dado que este caso foi durante o verão e que estava muito calor, durante o dia a transpiração não iria permitir uma boa absorção);
- nos 5 dias seguintes 1 vez por dia, aplicando dia sim - dia não;
- nos outros 5 dias aplicar 1 vez 1 dia, durante 2 dias não aplicar, voltar a aplicar 1 dia e acaba.

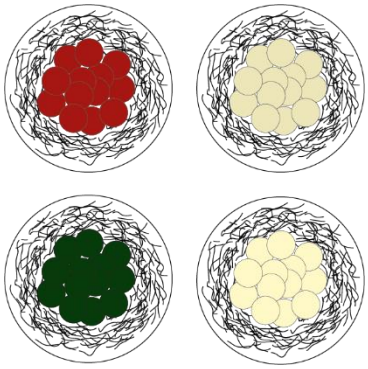
Desta forma, serão 15 dias de tratamento com corticosteroides, já a contar com o desmame. Aconselhei ainda a colocar o anticort® durante estes 15 dias, 2 a 3 vezes por dia conforme necessidade, mas principalmente nos dias em que não coloca o Elocom®. Além do tratamento, recomendei ainda, como forma preventiva e como algo que deve aplicar para o resto da vida, um bom creme emoliente, que vá reestruturar a camada lipídica da pele, de forma a prevenir o reaparecimento do eczema de contacto. Esta prevenção é essencial porque, dado que estas lesões já se tinham manifestado antes, é provável que a utente tenha uma pele mais atópica e suscetível de desenvolver este tipo de lesões. Alertei ainda a menina que, se passado os primeiros 5 dias do tratamento não visse nenhuma melhoria, deveria remarcar uma consulta com o dermatologista, porque nesse caso pode não ser um eczema de contacto, mas sim outra patologia com uma expressão aproximada.

Anexo III - *Overview* da análise SWOT do estágio em Farmácia Comunitária



Monografia

"Plant-mediated green synthesis of metal-based nanoparticles intended for dermopharmaceutical and cosmetic applications"



Resumo

A pele é a principal barreira que protege o corpo humano contra os fatores ambientais. Face ao aumento das patologias dermatológicas, tem havido um interesse crescente no desenvolvimento de sistemas eficazes para aplicação tópica. O maior desafio apresentado é o aumento da penetração dos ingredientes ativos através da barreira da pele, paralelamente à necessidade de obtenção de um nível de retenção cutânea suficiente capaz de atingir concentrações terapêuticas.

Os metais, em particular os metais nobres, são utilizados desde sempre para tratar e prevenir problemas de saúde, como por exemplo patologias dermatológicas. As nanopartículas têm sido extensamente usadas para aplicações tópicas, dadas as suas reconhecidas vantagens, nomeadamente o aumento da solubilidade de fármacos apolares, a possibilidade de libertação controlada, e o aumento da estabilidade, e capacidade para atingir áreas específicas e libertar concentrações de fármaco suficientemente elevadas. De forma a tirar partido das propriedades únicas das nanopartículas e das atividades biológicas dos metais, nos últimos anos têm sido sintetizadas várias nanopartículas metálicas, tais como nanopartículas de prata (AgNPs), ouro (AuNPs), zinco (ZnNPs), óxido de zinco (ZnONPs), cobre (CuNPs) e óxido de cobre (CuONPs). Estas nanopartículas metálicas são estruturas flexíveis que permitem controlar as suas propriedades físicas, com propriedades de superfície distintas que lhes permitem uma elevada aplicabilidade em dermofarmácia e cosmética. Os métodos convencionais para sintetizar nanopartículas (químicos e físicos) estão associados a desvantagens marcadas, sendo as mais preocupantes o elevado custo (em materiais e fontes, energia, tempo e espaço) e as toxicidades humana e ambiental. Desta forma, surgiu a necessidade de desenvolver uma via de síntese alternativa, nomeadamente a síntese *green*. De uma forma geral, a síntese *green* consiste no uso de fontes biológicas (plantas, bactérias ou fungos) para sintetizar nanopartículas seguras para o Homem e o ambiente. Com o desenvolvimento da síntese *green* têm sido utilizadas matérias primas alternativas, entre estas, as plantas.

A síntese *green* de nanopartículas mediada por plantas baseia-se no uso de extratos de plantas para sintetizar nanopartículas, e as suas vantagens notórias abriram caminho para desenvolvimentos significativos na síntese de nanopartículas, em detrimento da complexa e tóxica síntese química e física. As nanopartículas metálicas produzidas pela síntese mediada por plantas demonstram atividades biológicas reconhecidas, como, por exemplo, atividade anticancerígena, antioxidante, anti-inflamatória, antimicrobiana, cicatrizante e anti-envelhecimento. A avaliação da segurança das fito-nanopartículas metálicas é de extrema

importância, dada a falta de estudos toxicológicos e de problemas de conceção que muitos dos estudos existentes apresentam. No entanto, os estudos atuais sugerem a biocompatibilidade e segurança as fito-nanopartículas metálicas, bem como atividades biológicas relevantes e significativamente melhoradas. Face a este cenário, existe ainda um longo caminho a percorrer até que as fito-nanopartículas metálicas possam ser aplicadas no ramo médico, farmacêutico e cosmético mas, até à data, os estudos sugerem um enorme potencial para a sua translação clínica no âmbito das aplicações dermofarmacêuticas e cosméticas. Esta revisão foca-se na discussão das nanopartículas metálicas sintetizadas a partir de extratos de plantas, focando a sua produção, caracterização e atividades biológicas que suportam a sua aplicação tópica com finalidades dermofarmacêuticas e cosméticas.

Palavras-chave: Nanopartículas metálicas, nanotecnologia, síntese *green* mediada por plantas, extrato de plantas, prata, ouro, tópico, fitocomponentes, antimicrobiana, antioxidante, anticancerígena, cosméticos, dermofarmacêuticos.

Abstract

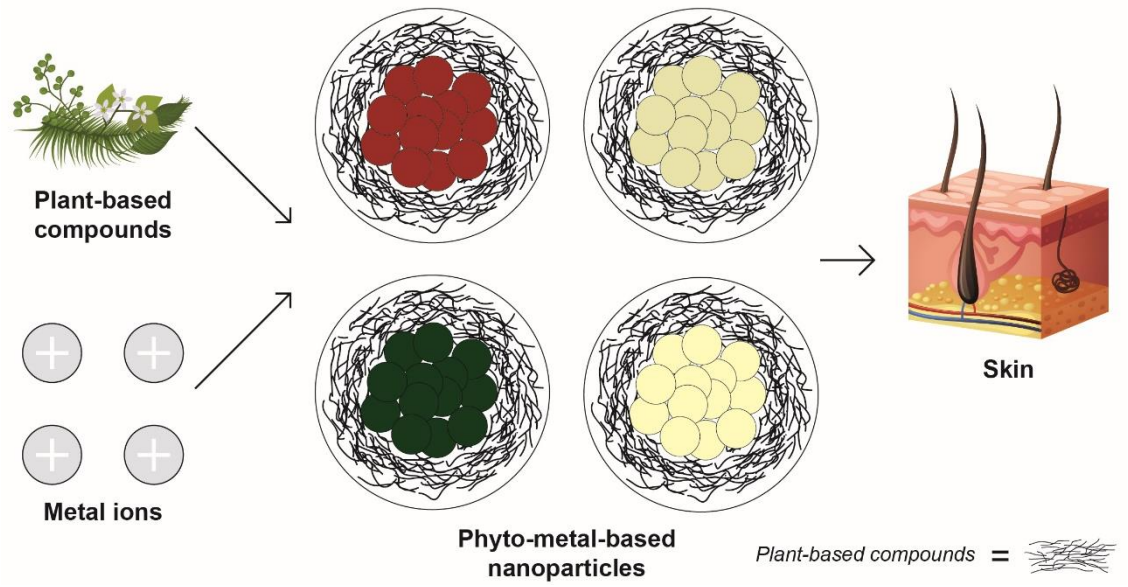
The skin is the primordial barrier that protects the human body against environmental factors. Due to the arise of dermatological pathologies, a crescent interest in the development of efficient systems for topical applications has been verified. The highest challenge consists of increasing the penetration of the active ingredients through the skin barrier, alongside to the need of obtaining enough retention in order to achieve therapeutically concentrations.

Metals, specially nobble metals, have been used for years to treat and prevent health issues, among them dermatological disorders. Nanoparticles have been extensively used for topical applications given their advantages, namely the enhanced solubility of apolar drugs, the possibility of controlled release, the higher stability and the capability to target specific areas and delivery of high active ingredients concentrations. In order to take advantage of the before mentioned unique properties of nanoparticles and the biological activities of metals, various metal-based nanoparticles have been synthesized in the past few years, such as silver (AgNPs), gold (AuNPs), zinc (ZnNPs), zinc oxide (ZnONPs), copper (CuNPs) and copper oxide (CuONPs) nanoparticles. These metal-based nanoparticles are flexible structures that allow the control of physical characteristics, with enhanced surface properties, which provides a high applicability in dermopharmacy and cosmetics. The conventional methods for synthesizing nanoparticles (physical and chemical approaches) are associated with major drawbacks, being the most concerning the high cost (in resources, energy, time and space) and human/environmental toxicity. Hence, the need to develop an alternative synthesis pathway was imposed, giving rise to the green synthesis. In general, green synthesis consist of using biological sources (plants, bacteria or fungi) to synthesize ecological benign and non-hazard nanoparticles. With the development of green synthesis, starting materials have been used more frequently, among them plants.

Plant-mediated green synthesis of nanoparticles is based on the use of plant extracts to synthesize nanoparticles, and their outstanding advantages have paved the way for exciting developments on nanoparticle synthesis to the detriment of complex and toxicity-associated chemical and physical synthesis. Metal-based nanoparticles produced by plant-mediated synthesis demonstrate notorious biological activities, i.e., anticancer, antioxidant, anti-inflammatory, antimicrobial, wound healing and antiaging activities. Safety assessment of phyto metal-based nanoparticles (phyto-MNPs) holds significant importance due to the lack of toxicological studies and the conception issues that some of the available studies hold. However, current studies suggest the biocompatibility and safety of phyto-MNPs, together with significantly improved and relevant biological activities. Against this backdrop, there is still

a long way to run until the application of phyto-MNPs in the medical, pharmaceutical and cosmetic fields, but studies so far show a very high potential towards their clinical translation for dermopharmaceutical and cosmetic applications. This review focuses on metal-based nanoparticles synthesized resorting to various plant extracts, including their production, characterization and the biological activities that support their topical application for dermopharmaceutical and cosmetic purposes.

Graphical abstract



Keywords: Metallic nanoparticles, nanotechnology, plant-mediated green synthesis, plant extract, silver, gold, topical, phytocompounds, antimicrobial, antioxidant, anticancer, cosmetics, dermopharmaceutical.

List of abbreviations

AFM, Atomic force microscopy

AIF, Apoptosis-inducing factor

ATP, Adenosine triphosphate

B16, Mouse melanoma cell line

B16F10, Human skin carcinoma cells

Bax, Bcl-2 associated X

BHT, Butylated hydroxytoluene

CO, Carbonyl

DHFR, Dihydrofolate reductase

DI-H_20, Deionized water

DLS, Dynamic light scattering

DNA, Deoxyribonucleic acid

ECM, Extracellular matrix

EDAX, Energy dispersive X-ray spectroscopy

FTIR, Fourier transform-infrared spectroscopy

GSH, Glutathione

HaCaT, Human epidermal keratinocytes

HDF, Human dermal fibroblast

HMVEC, Human Microvascular Endothelial

HUVEC, Human umbilical vein endothelial

LDH, Lactate dehydrogenase

LPO, Lipid peroxidation

L929, Mouse fibroblast

MNP, Metal-based nanoparticles

MHDF, Normal human dermal fibroblast

NiH3T3, Normal fibroblasts

NP, Nanoparticle

NTA, Nanoparticle tracking analysis

OH, Hydroxyl

PCNA, Proliferating cell nuclear antigen

PCR, Polymerase chain reaction

PDI, Polydispersity index

Phyto-MNP, Phyto metal-based nanoparticles

ROS, Reactive oxygen specie

RT-PCR, Reverse transcription polymerase chain reaction

SC, Stratum corneum

SEM, Scanning electron microscopy

SPF, Sun protection factor

TEM, Transmission electron microscopy

UV, Ultraviolet

UV-VIS, Ultraviolet-visible

XRD, X-ray diffraction

1. Introduction

Nanotechnology is a promising and quickly evolving field in science that manipulates matter in the nanoscale range, accounting for outstanding contributions to technological innovation so far, including the development of tailorable nanoscale materials - nanomaterials - evidencing improved features and innumerous applications within the biomedical field [21, 28, 31, 36]. Nanomaterials are known to exhibit enhanced properties when compared to larger-scale materials, such as distinct optical properties and enhanced chemical reactivity [28]. Among the distinct nanomaterials developed so far, nanoparticles have attracted a lot of enthusiasm owing to unique characheristics, particullarly the capablity of modifying the catalytic, thermal and mechanical properties and the increased surface-volume ratio [48]. Furthermore, the interest in nanoparticles can also be a result of their vast array of applications and tailorable physicochemical properties, such as size, morphology, surface charge and stability [11, 19]. These properties have made nanoparticles very interesting in pharmaceutical and medical areas, for enhanced drug delivery, drug controlled release and diagnostic applications, and even in cosmetic field [28, 44, 47].

Metal-based nanoparticles are entities composed of metals (metal nanoparticles) or metallic compounds (metal oxide nanoparticles) [39, 44]. Thanks to their noteworthy features including the generally spherical shape, small size, metallic composition and high surface area, metal-based nanoparticles have been broadly used in many science applications such as biological and medical fields. Not to mention their utilization in optical, electronic, magnetic, engineering and plasmonic areas [7, 11, 32, 33, 47]. Subsequently, metal-based nanoparticles have been utilized for a range of applications, including biomedical purposes (cancer diagnosis and therapy, delivery systems for antitumor agents, photodynamic and photothermal therapies, wound healing, cosmetics, among others) [7, 11, 44, 47] and non-biomedical purposes (bio-chemical sensing, O_2 storage capacity, CO_2 solar conversion applications, catalysis, water treatment, paints, optoeletronics, photography, photonics, information storage) [7, 11, 50].

Different large-scale methods for preparation of metal-based nanoparticles have been described so far, either chemical (sol-gel method, polyol synthesis, chemical reduction, precipitation) or physical methods (combustion assisted by microwave, pulsed laser deposition, and laser evaporation) [11, 47]. Despite their extensive use, chemical and physical methods are associated with several drawbacks, namely use of toxic solvents and chemicals, production of dangerous by-products, high cost and low yield [11]. Although chemical synthesis is a common path to synthesize metal-based nanoparticles, it is (i) very expensive,

(ii) often recurs to toxic chemicals as capping, stabilizing or reducing agents, and (iii) results in toxic chemicals absorbed at the surface - which perturb biocompatibility of metal-based nanoparticles (MNPs), demanding complex steps and generate non environmental-friendly byproducts [11, 42, 46]. Laser evaporation, for instance, depends on refined and expensive instruments, convenient handling and is related to low production yield [11].

Thereby, there is an urgent need to develop a one-step, cost-effective, reliable, non-toxic and biocompatible way to synthesize eco-friendly metal-based nanoparticles, which raises attention to biological methods applying enzymes, microorganisms and plant extracts [11, 32, 42, 46, 47]. Consequently, researchers have shown an increased interest in green synthesis, as it uses harmless solvents and reducing agents, and sustainable procedures for synthesizing metal-based nanoparticles [19, 33, 46].

Green synthesis features improvements over physical and chemical methods, since it overcomes the setbacks of conventional methods, arising as a clean, simple, viable and biocompatible procedure [33]. Besides, it is considered ecologically and environmentally benign, as well as using convenient and available bio-resources[1] which act as reducing and capping/stabilizing agents. Furthermore, it is easy to scale-up, free from undesired byproducts, and recurs to harmless and universally accepted solvents (e.g. water) and a moderate reaction medium. In addition, fewer purification steps are needed and there is no demand to utilize aggressive procedures such as high pressure or vacuum conditions, high energy, sophisticated instrument or toxic chemicals [11, 28, 32, 46, 47].

Among all the methods used in green synthesis of MNP (phytological, phycological, mycological and bacteriological fabrication), the plant-based synthesis emerges as the best choice since it produces stable NPs, in a quick and stable synthesis [28, 42, 46, 47]. This can be explained by all the benefits of using plant extracts, for instance their easy accessibility, safety to handle, vast range of metabolites, elaborated metabolic pathways, and stable and quicker synthesis that generate stable, benign and side effects-free metal-based nanoparticles [11, 19, 42, 46, 47]. Plant-based synthesis are more valuable than microbe-based synthesis due to elimination of biohazard, time-consuming and elaborated isolation procedures. Additionally, it avoids development of cell cultures and maintenance and can avoid high costs since it can be narrowed for non-aseptic large productions and circumvent the arduous surface modification steps and the time consuming process of microbial screening and vector's building [11, 19, 28, 46, 47]. Till this date, a broad range of plants have been used for synthesis of metal-

[1] Fungi, microorganisms, algae and plants.

based nanoparticles [11, 47]. Medicinal plants have been extensively used thanks to their phytochemicals bearing high therapeutic value [46].

Plant extracts consist of numerous phytochemicals, such as flavonoids, phenolic acids, alkaloids, saponins, carbohydrates, amino acids and proteins, and terpenoids, that play a vital role in synthesizing metal-based nanoparticles from metallic ions, as they act as capping/stabilizing and reducing agents [10, 11, 42]. Plant-mediated metal-based nanoparticles displayed activity against genotoxicity, changes related to apoptosis and oxidative stress and feature antimicrobial, anticancer, antioxidant, anti-inflammatory, antidiabetic and antiviral activities [11].

In the past few times, the biggest pharmaceutical companies have been directing their efforts to develop innovative therapies to treat skin diseases [44]. Nowadays, one of the major concerns are cutaneous infections [44]. In particular, wounds are an important matter of study, since wound chronicity usually leads to sepsis, resulting in the death of the patient [40]. Obviously, the multidrug resistance of several microorganisms has also been a major problem [16]. This way, newly formulated wound healing agents, together with antibacterial and antifungal abilities, have been emerging [44]. Skin cancers are on the arise worldwide, so a tremendous effort has been made to develop formulations for prevention and treatment [15, 16, 24, 37]. Besides the treatment of dermatological diseases, there has also been a growing interest in the cosmetics field, especially in the anti-aging, sunscreens and moisturizers areas. As well known, sunlight leads to skin ageing, but it is also a major cause of skin cancer; consequently, sunscreen formulations have been one of the most extensively studied [15, 20].

Skin is the main protection of the body against external factors, and the stratum corneum (SC) (lipid matrix) performs a key role [18]. Given their potential to overcome the SC of skin, nanoparticles have been investigated to be used in topical formulations [27]. Metal-based nanoparticles are a result of an effort to take advantage from both metals and nanoparticles. Subsequently, the biological activities of metals are combined with the enhanced properties of nanoparticles which, together with the benefits of plant-mediated green synthesis and the activities of plant extracts, makes the plant-mediated metal-based nanoparticles a valuable material for topical applications [3, 5, 17, 31].

Notwithstanding the vast biomedical applications described so far for plant-mediated metal-based nanoparticles, this paper will focus on plant-based green synthesis of metal-nanoparticles for topical applications, in both dermopharmacy and cosmetics.

2. Plant-mediated green synthesis of metal-based nanoparticles

Green synthesis of MNPs stands as a production method that uses water as solvent and eco-friendly starting materials, resulting in functional and biocompatible nanoparticles [20, 23]. Among the green methods for synthesizing MNPs (microorganisms or plant extracts as a starting material), the plant-based synthesis proves to be more convenient, given that microorganisms require a lot of effort and attention [37] and plat-based synthesis does not need a special reducing or capping/stabilizing agent [25].

2.1 Preparation of plant extracts

Several plant parts can be used to produce the plant extract, such as leaves [6, 7, 21, 30, 44, 46], peel [23], fruit [19], seeds [17, 27], petals [35], rhizome [35], aerial parts [7], or even the whole plant [5]. The extract production follows a general protocol, that can have slight modifications [35]. The plant parts can be used dry [27, 30, 37] or fresh [6, 19, 21, 44].

If the plant part is fresh, there is the need to a washing pretreatment , generally followed by drying [5, 6, 19, 21, 32, 44, 46]. Once the plant part is washed and dried, the part is reduced by grounding, finely cutting or pulverizing. Then, extraction is done by boiling the diminished plant parts with a certain volume of Milli-Q or deionized water; sometimes, instead of boiling, the extraction is made in a Soxhlet apparatus [21, 32]. Finally, the residues are removed by filtration [6, 17, 23, 30, 43, 46], but the extract can also be concentrated by using a rotary flash evaporator [21]. Normally, the extract solvent is water, in order to produce water extracts, because of its harmlessness [45]. However, it is possible to use other solvents, for example methanol or ethanol, which demands an extra step to eliminate the solvent, for example vacuum distillation (evaporation under reduced pressure) or drying employing a water bath and, generally, a step to dissolve the dried extract in water, thus producing a aqueous extract [1, 7, 32].

The plant extracts comprise a complex arsenal of biomolecules, therefore reduction process is expected to be an interplay of various active components and their functional groups / synergetic mechanisms [19, 23, 29, 46]. It is important to note that these bioactive ingredients are not evenly distributed along the structure of the plant, so their content varies between different parts [6, 17, 19].

Plants contain various active phytocompounds, which can be divided in primary and secondary metabolites. The primary metabolites are vitamins, proteins, amino acids, nucleic acids, polysaccharides (e.g. pectins) and reducing sugars. All the other biomolecules present in the extract consist of secondary metabolites, such as alkaloids (e.g. quinines), terpenes (like

triterpenes and terpenoids such as carotenoids), glycosides (e.g. saponins and coumarins) and phenolic compounds (flavonoids, tannins and phenolic acids) [17, 19, 23, 29, 44, 46]. The secondary metabolites possess a lot of key biological properties, like antibacterial and antifungal [32, 44], anti-inflammatory [43], antioxidant [19, 23, 43, 44] and anticancer [19, 32]. Furthermore, other insightful activities are observed, such as enhancement of immune function / immunomodulation [43], collagen production, skin protection against damage caused by ultraviolet (UV) rays, anti-aging [19] and treatment of psoriasis [32]. Therefore, the synergy between the activities of secondary metabolites and MNPs provide to the phyto-MNPs biocompatible capabilities toward diversified biomedical applications [32, 44]. Secondary metabolites are organic compounds that do not participate directly in cellular growth and evolution of plant, that are produced to handle any stress circumstance affecting the plant and have been proven to have the ability to reduce the metal ions [29]. Although primary metabolites have a key role in the production, secondary metabolites shows up to be the main agents in the synthesis of MNP, acting as reducing, stabilizing and capping agents, reducing the metallic ion, in one step, to zero valence MNP [6, 17, 23, 29, 44, 46]. The robust coating created on MNPs renders them stable against agglomeration and aggregation [19, 29, 44, 46].

2.2 Production of plant-mediated metal-based nanoparticle

The production of plant-mediated metal-based nanoparticles consists of two main steps: (i) reduction of the metal precursor and (ii) stabilization of the synthesized MNPs [17, 23]. The synthesis of phyto-MNPs initiates with the (i) formation of a chelate between the metal and the hydroxyl (OH) groups of phyto-components, followed by oxidation of OH groups, into the analogous carbonyl (CO) groups, leading to reduction of metal ions - nucleation stage. Next, there is the growing stage, where the extract acts as (ii) stabilizing agent, by means of coating the MNPs to stabilize them and prevent their size from keeping getting bigger. Plant extracts are powerful capping agents, stabilizing the phyto-MNPs via repulsive forces, which include electrostatic interactions and/or steric hindrance, leading to high stability [17, 23, 44, 46].

During the synthesis of phyto-MNPs, phyto-constituents might get adsorbed onto the surface of MNPs or assemble an organic matrix that integrate them, in a way that MNPs are embedded towards the system. Typically, negatively charged functional groups, such as hydroxyl groups of polyphenols, are physical connected to the MNPs' surface, leading to a surface charged negatively, yielding highly stable phyto-MNPs. This attachment happens by the partial binding of oxygen atoms and the release of chemisorbed protons of the surface by

virtue of a process of electron transfer. This process performs a major role in the change of the surface behavior and contributes to negative zeta potential, that correlates with the colloidal stability of the phyto-MNPs [19, 23, 29, 43, 44].

The FTIR spectroscopy is a technique utilized to identify the functional groups present in the biomolecules of the extract as well as in the synthesized phyto-MNPs, and provides useful information for comprehending the role of each functional group in the synthesis of the phyto-MNPs.

The presence of certain compounds correlate with the presence of specific organic biomolecules, such as: terpenoids (alcohols, ketones, aldehydes and esters), carbohydrates (alcohols), polyunsaturated fatty acids (carboxylic acids), enzymes, proteins and amino acids (amides, carboxylic acids, amines), phenolic compounds / polyphenols such as phenolic acids, flavonoids and tannins (alcohols, phenols, carboxylic acids, aldehydes, ketones, esters, ethers), flavonoids and alkaloids (heterocyclic compounds), tannins, phenolic glycosides and reducing sugars (ethers, alcohols) [21, 29, 32, 35, 44, 46].

Terpenoids, carbohydrates, glycosides and vitamins possess functional groups that have a role in reduction, surface coating / capping and subsequent stabilization [1, 19, 29, 30, 44-46]; phenolic compounds such as flavonoids, tannins and phenolic acids have a central role in the bioreduction, but can also act as capping and stabilizing agents[2] [19, 29, 30, 42, 44, 46]; proteins and amino acids are strongly bound to MNPs, capping / coating and protecting them from agglomeration, thereby stabilizing the phyto-MNPs [32, 35, 44, 46]; alkaloids are powerful reducing agents [1, 44]. From this analysis, it can be stated that the main functional groups responsible for the synthesis of phyto-MNPs are: hydroxyl groups, carboxylic groups, carbonyl groups and amine groups. Hydroxyl groups are directly involved in the synthesis and are mainly responsible for the bioreduction, since they work as ligands, being able to bind with metal ions [1, 7, 29, 44, 46]. Carboxylic groups also have the ability to bind with metal ions, so they may act as reducing agents and as surfactants that coat the MNPs by attaching on their surface, leading to their stabilization [35, 44, 46]. Free amino groups and carbonyl groups have a robust binding ability for the metal, so they act as reducing agents - since the majority of the phytocompounds are apolar, and over the course of synthesis the water is the main solvent, the amino groups are free and can bind with metal atoms, thereby reducing them - and capping agent - helping to form a coat, avoiding agglomeration and providing stability [32, 35, 44, 46].

[2] For example, flavanones have a role in the surface coating.

Both the major classes and functional groups responsible for the phyto-synthesis of MNPs are represented in Figure 1.

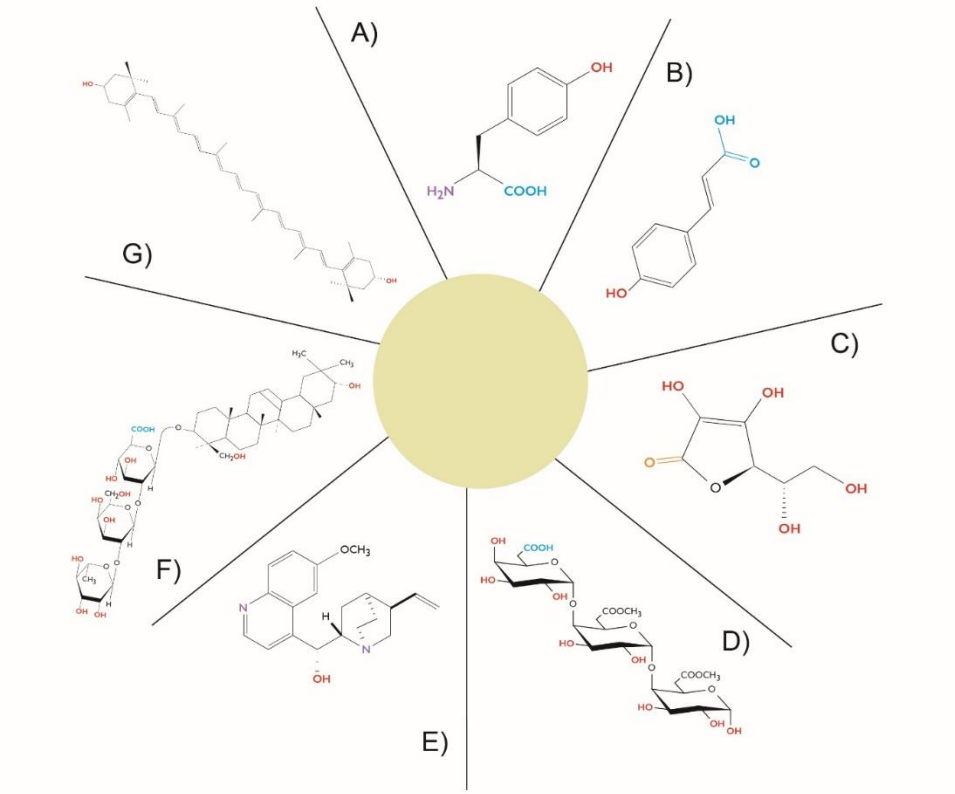


Figure 1 - Relevant classes of plant-based compounds in phyto-synthesis of metal-based nanoparticles. The most important classes are represented by: A) Tyrosine (amino acid), B) p-Coumaric acid (phenolics), C) Vitamin C (vitamins), D) Pectin (polysaccharides), E) Quinine (alkaloids), F) Saponin (glycosides), G) Carotenoid (terpenes). The main functional groups responsible for the synthesis are represented by colours: hydroxyl group at red, carboxyl group at blue, carbonyl group at orange and amine group at purple.

Plant-mediated green synthesis of metal-based nanoparticles is based on a simple, rapid and one-pot method, which may have slight modifications [19, 26, 38]. Briefly, (i) the plant extract is mixed and homogenized with the precursor metal salt solution, normally at room temperature, under continuous stirring, followed by (ii) purification and concentration of the extract [2, 21, 44, 48] (Figure 2).

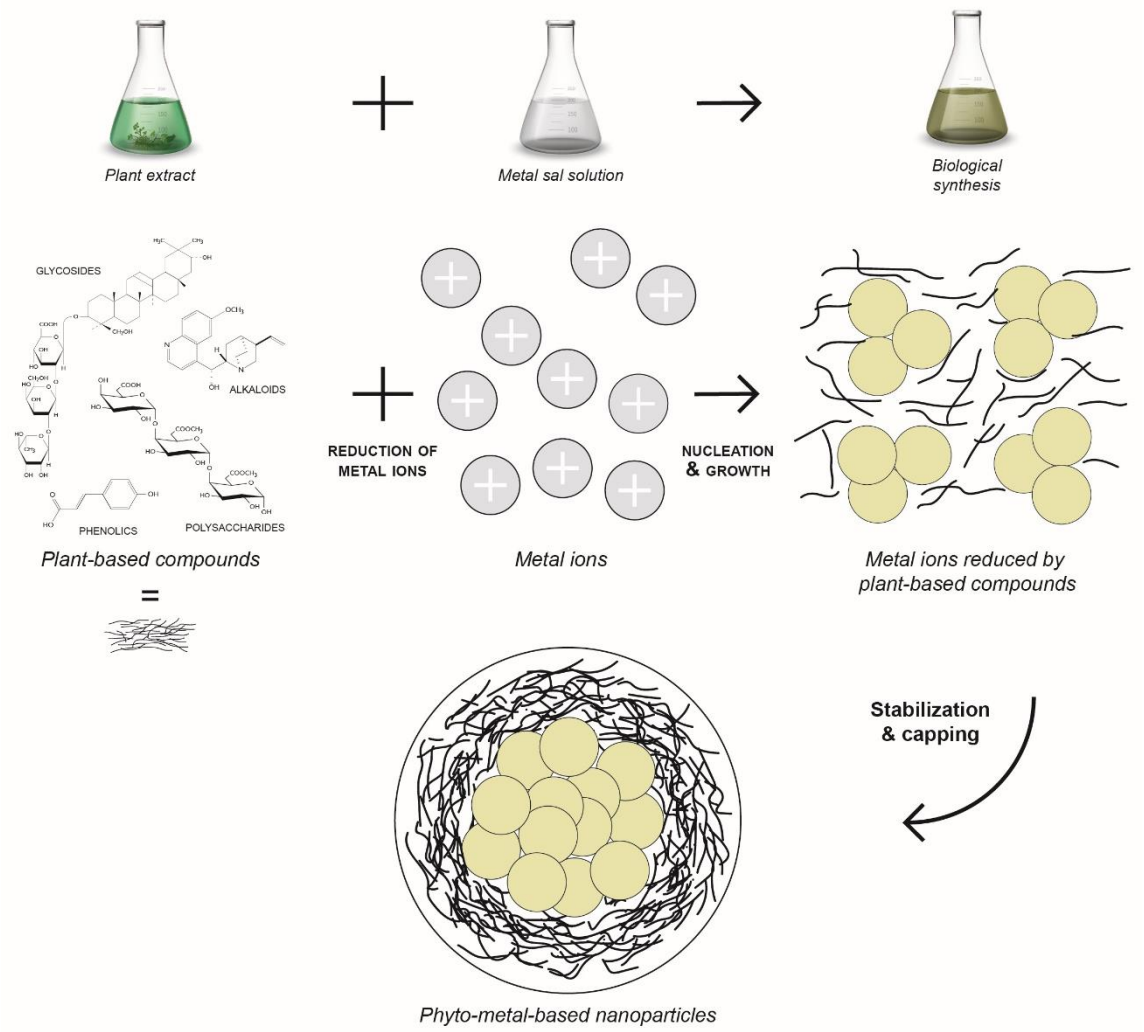


Figure 2 - Schematic view of plant-mediated synthesis of metal-based nanoparticles, using phyto-AgNPs for exemplification.

Typically, the salt solution is aqueous [25, 30, 42, 50] and it might be in the form of nitrate solution - e.g. silver, zinc, copper and cerium [2, 21, 30, 50]; acetate solution - e.g. zinc and copper [42, 48]; chloride solution - e.g. gold and platinum [7, 25, 34]; or sulfate solution - e.g. copper [41]. In some cases, the extract is heated before the mixture with the precursor salts [48], but normally the homogenization is done in a water bath or a magnetic hot plate [1, 2, 35, 37]. Sometimes, pH can be adjusted to optimize yield of the protocol [15, 23, 28, 43]. In order to prevent photoinduced phenoms, the mixture may be protected with foil [30]. After stirring, the mixture can be treated recurring to two different methods: (i) centrifugation and washing of the pellets (with deionized, distilled or Milipore water), that can be done 2 - 3 times to remove any biomolecules of the extract that are water-soluble and, sometimes, there is the need to wash it again with ethanol [46], occasionally followed by drying of the pellets and grind or lyophilization into a fine powder [2, 6, 23, 35, 43, 46, 48]; (ii) dry in an oven,

washing of the powder and regular heat treatment [21, 30]. After this procedure, phyto-MNPs are stored in airtight containers [21] or simply in a vial [28].

Some modifications can be made, for example, in both methods, filtration can be added before [21] or after washing [2]; another example is the purification technique, that usually is centrifugation, but can also be done by PLC or ion exchange chromatography [13].

Without optimization, the synthesized MNPs are heterogeneous and the production presents low yield; this way, optimization is key to achieve a high yield and stable production of capped and homogenous phyto-MNPs [1, 15].

2.3 Critical factors to plant-based production of metal-based nanoparticles

Various production factors are key to the optimization of phyto-MNPs synthesis, affecting the yield[3], the agglomeration state and characteristics of the synthesized phyto-MNPs[4] [6, 17, 32, 35, 48]. Critical factors involving the plant extract, such as the plant part utilized for extraction [5], concentration of plant extract [5, 7, 15, 32], composition of the extract [7, 48], molecular weight of biomolecules [29, 32], capping agent [5, 21, 43] and storage conditions of the plant extract (such as aging time of the extract and temperature) [17] must be considered to achieve an optimal synthesis. Additionally, precursor metal salt concentration [2, 6, 15, 25, 48], metal type [48] and ratio of plant extract and metal solution [5, 6, 15, 25] also play a vital role in the synthesis. Furthermore, there are some external factors that need to be ensured to achieve viable production, as doping's concentration [21], temperature [2, 6, 15, 25, 35], pH [15, 17, 23, 35], different solvents [2, 7], reaction time [2, 6, 35], light [17], dissolved oxygen [17, 35] .

2.3.1 Concentration of plant extract

Variations in the concentration of plant extract leads to changes in the absorption intensity, size, shape, dispersity and agglomeration of phyto-MNPs. A decrease in the concentration of plant extract leads to bigger size, which correlates with a red shift in the SPR peak [7], while an increase leads to smaller, spherical and isotropic phyto-MNPs [5, 7, 22, 27], with good dispersity and no agglomeration [10, 27]. A great example is the study of Lin *et al.* [27], in which the phyto-AuNPs synthesized with higher concentration of plant extract present a size lower than 15 nm and a spherical shape, compared with a size >100 nm and an hexagonal

[3] Key role in NPs growth rate.
[4] Absorbance intensity and wavelengths, color, size, shape, stability, zeta potential, charge surface roughness, crystallinity.

shape for the phyto-AuNPs synthesized with lower concentration of plant extract. Decreasing the concentration of plant extract also diminished the availability of phytocompounds[5] for capping, resulting in aggregation and, consequently, larger phyto-MNPs [27]. Moreover, just like the reaction time, in a certain interval, an increase leads to higher absorption intensity, which means higher reduction yield (and, consequently, lower reaction time) [1, 7, 10, 32].

2.3.2 Capping agents

Capping agents, besides the protection role, can also influence the elemental composition and size of phyto-MNPs. They alter the content of elements in the phyto-MNPs [21, 43], lead to higher hydrodynamic size [43] and create an additional shell that protects phyto-MNPs, making it difficult for them to be captured by macrophages, thus improving pharmacokinetics and bioavailability and decreasing clearance [43]. Furthermore, surface-attached phytochemicals lead to an increase in the surface roughness [5].

2.3.3 Chemical composition of the plant extract

Chemical composition of the extract has been suggested to impact the shape, size and rate of the reduction of phyto-MNPs. An example is that higher antioxidant activity leads to larger size of phyto-MNPs, and this can easily be explained by the fact that higher antioxidant activity results in a quick generation of small phyto-MNPs which, with the assistance of phytochemicals, grow into bigger phyto-MNPs [1, 5, 12, 35, 46]. Given that the plant composition varies among the plant parts, the part chosen for the extract will also impact the size of phyto-MNPs; an example is the study of Appapalam and Panchamoorthy [5], where phyto-AgNPs synthesized using leaves were significant smaller (<15 nm) compared to phyto-AgNPs synthesized with flowers (43.5 nm). Another example is the importance of the solvent used for the extraction, since it dictates which fraction of the extract (polar or apolar) will produce the phyto-MNPs, which for its part correlates with their shape - for example: polar fraction, including aldehydes, carboxylic acids and ketones produce mainly triangular structures [7]. The molecular weight of the biomolecules should also be considered, since the lower it is, the bigger the influence in the synthesis [29, 32].

[5] Such as polyphenols.

2.3.4 Storage conditions of the plant extract

The storage conditions of the extract have a major influence in the size, crystallinity, dispersity and morphology of the phyto-MNPs and in the chemical composition of the extract. While in the storage, the hydrolysis of phytocomponents may lead to bacterial growth and loss of stabilization properties, resulting in bigger size of phyto-MNPs (which explains the increase in size with the aging time of plant extract). In phyto-MNPs synthesized using extract that has been stored at room temperature, with increased aging time of the plant extract there is an increased absorption of the peak at maximum wavelength and a slightly decrease in the wavelength; while in phyto-MNPs synthesized using extract that has been stored in the cold, both the wavelength and absorbance increases with the increase of aging time. Increasing the storage temperature and the aging time of the plant extract, decreases its capability of stabilization, resulting in larger size and bigger polydispersity; however, the opposite happens when the storage temperature and aging time of the plant extract decreases [17].

2.3.5 Metal salt

The type of metal salt and its concentration affects the color of the reaction mixture and the size and shape of phyto-MNPs. It has been found that higher concentrations of metal salt leads to bigger size of phyto-MNPs [2]; however, addition of silver or silver salt to another phyto-MNP or to the medium seems to decrease the size of phyto-MNPs, compared with the other phyto-MNPs alone [48].

2.3.6 External factors

Doping correlates with the characteristics of phyto-MNPs, as higher doping concentration results in higher surface area and grain size, but lower particle size [21]. External factors such as light, pH and dissolved oxygen can degrade the active compounds, affect the dispersity and the size of phyto-MNPs [17].

The pH is an absolute key factor, affecting the reducing ability of phytoconstituents, release of the protons from the surface of NPs, size, zeta potential values, position and intensity of the peak of phyto-MNPs. At acidic conditions, the functional groups on the surface of phyto-MNPs are present in the protonated form, leading to positive zeta potential values, poor stability and aggregation [7, 15]; also, acidic pH results in heterogeneous phyto-MNPs population and bigger size distribution [15]. Basic conditions are necessary for the phytoconstituents of the extract act as reducing and capping agents, since the release of chemisorbed protons of the NPs' surface is only possible at basic pH values [10, 23, 44]; in

addition, with the increase of the pH, there is an increase in the peak's intensity, however it may lead to a shift of the maximum absorbance peak position, beyond certain value [10, 23].

Oxygen levels and reaction time affect the reactivity and the yield of the produced phyto-MNPs. The control of the reaction time allows the achievement of various sizes [10, 35]; in its turn, the presence of oxygen results in slow nucleation of ions and growth of MNPs, which might disturb the morphology, size and crystallinity of phyto-MNPs [35].

Temperature plays a central role in the formation, stability, size, shape and in the peak's intensity of phyto-MNPs [35]: decreasing temperature leads to a decrease in the production rate of phyto-MNPs [25], while increasing temperature results in an increase in the peak's intensity and a decrease in the reaction time which, in turn, leads to smaller size [10, 19]. Temperature variations will affect shape and, consequently, crystalline nature - for example, phyto-MNPs annealed at 500°C contain a population of small rods, that might contribute to the polycrystalline nature, contrary to amorphous phyto-MNPs dried at 100°C [30].

In most of the studies, the reduction of metal ion to phyto-MNP has been assessed in two different ways: color transformation and specific UV absorption [5]. Ultraviolet-visible (UV-VIS) spectroscopy is one of the most practical ways to monitor the formation of MNPs because these particles display surface plasmon resonance (SPR) properties, which leads to a characteristic band [6]. Furthermore, the kinetics of the reduction may be studied through UV-VIS absorbance in relation to time [19]. The color change approach was employed since it is a result of SPR and is characteristic of each metallic nanoparticle [3, 12, 26].

There is the urge to remember that both the UV absorption and the color change are affected by a number of factors, including factors that affect the production and the characteristics of phyto-MNPs, which explains the discrepant results often found in research articles. These factors include, among others, the size of the phyto-MNPs, the concentration of plant extract, presence of impurities and the medium pH.

Table I - Synthesis confirmation techniques: color and wavelength range of different metal-based nanoparticles.

Metal-Based Nanoparticle	Wavelenght (nm)	Color	Reference
AgNP	400 - 460	Yellowish - Brown	*
AuNP	510 - 580	Ruby red	*
ZnONP / ZnNP	300 - 400	White / pale yellow	[2, 21, 28, 30, 44, 47, 49]
CuONP / CuNP	220 - 340	Dark green	[33, 42]

Abbreviations: NP, Nanoparticle;

* These intervals and colors are the result of the analysis of all the articles cited in the table 2.

3. Phyto metal-based nanoparticles

This review focuses its attention on metal-based nanoparticles, synthesized by plant-mediated route, and their dermopharmaceutical and cosmetic applications. The studies analyzed comprise plant-based MNPs, their properties and characteristics, their biological activities and the respective underlying mechanism, and the critical points regarding these activities. Among all the activities presented by phyto-MNPs, this article discussed only the activities that represent potential for topical applications.

A variety of spectral and microscopic methods are used to assess the characteristics of the synthesized phyto-MNPs [5, 32]. As stated earlier, UV-VIS spectroscopy is used to monitor the progression of the synthesis of the phyto-MNPs [35]. The majority of the studies used scanning electron microscopy (SEM) and transmission electron microscopy (TEM) to assess size and shape, energy dispersive X-ray spectroscopy (EDAX) to obtain the elemental composition [5], X-ray diffraction (XRD) to analyze the diffraction peaks in order to establish the crystalline nature [22] and Fourier transform-infrared spectroscopy (FTIR) to assess the functional groups [8] and to determine the potential biomolecules accountable for coating the surface (stabilizers) and for work as reducing agents [22].

Some studies also carried other analyses like dynamic light scattering (DLS) to determine the average hydrodynamic diameter and the zeta potential, which allows to infer the stability of phyto-MNPs concerning the surface charge [5, 8, 35], and the polydispersity index (PDI) [6]; nanoparticle tracking analysis (NTA) to assess the size in suspension [22]; atomic force microscopy (AFM) to analyze the surface roughness [5] and chromatography to identify the compounds in the extract [11, 31].

A discussion will be performed next regarding the dermopharmaceutical and cosmetic applications of phyto-MNPs, in particular AgNPs, AuNPs, Zn and ZnONPs and Cu and CuONPs, which is schematically depicted in Figure 3. Although phyto-PtNPs and phyto-

CeO_2NPs have potential for topical applications, they will not be part of this review, given the lack of studies about their phyto-synthesis and activities [25, 50].

3.1 Phyto silver nanoparticles

Silver is a noble metal with outstanding properties, among them the biocidal potential, in particular against multirresistant microorganisms, such as bacteria, and wound healing activities [1, 5, 8, 12, 14, 34, 48]. For this reason, silver and silver-based topical dressings have been used as a substitute antimicrobial agent to treat infections in chronic ulcers and open wounds, as a healing agent, and as a host protector from discoloration and oxidation [5, 8, 12, 14, 19, 23].

Phyto-AgNPs display exclusive optical, catalytic and electrical properties [6, 17, 26, 38, 46, 48], besides exceptional characteristics including oxidative resistance [5, 12], considerable surface area for attaching drugs and other components (e.g. ligands) [5, 10, 34, 38], high stability and surface reactivity [5], ease toward surface modification [1] and inertness [5]. These properties, together with their biocompatibility, endow phyto-AgNPs with innumerous advantageous attributes toward several applications in bioengineering and biotechnology [5, 12, 17, 34]. Among all the biological activities of phyto-AgNPs, some should be referred given their relevance in dermopharmacy, to treat dermatological diseases, and in cosmetic industry. The antimicrobial, antioxidant, anti-inflammatory and anti-angiogenesis activities make phyto-AgNPs ideal to be used in antiseptic sprays, wound care formulations and in surgeries [1, 5, 6, 8, 14, 17, 19, 34, 43]; additionally, the antioxidant and anti-aging activities place these phyto-MNPs in cosmetic industry [1, 12, 19, 22]. In addition, given their anticancer activity, phyto-AgNPs have been pointed out as suitable nanostructures for directed drug delivery [1, 19, 32, 35, 43].

3.1.1 Antimicrobial activity

Phyto-AgNPs possess antimicrobial activity over bacteria, protozoa, fungi and viruses, and this activity includes the inhibition of the biofilm production [3-5, 10-13, 23, 26, 32, 35]. This activity is achieved even at low doses and matches or even exceeds the activity presented by conventional antibiotics [12, 17]. The antimicrobial activity of phyto-AgNPs stems from a multisided mechanism: adhesion to microbial cells, penetration or inhibition of cellular transduction [17]. The (i) release of the phytocomponents attached to the phyto-AgNPs

surface[6] in the infected environment is one of the most important antimicrobial mechanisms [1, 4, 5, 11, 19, 23, 35, 43, 46, 48]. The phytocomponents intercalate into membrane bilayers composed of phospholipid and may bind to DHFR enzyme[7], disrupting bacterial key processes of cytoplasmatic membrane [43]. A further explanation for the antibacterial ability consists on the (ii) stress-related mechanism. The internalized phyto-AgNPs may generate Ag ions that reduce the catalase[8] level and converts free glutathione[9] (GSH) to its oxidized form (GSSH). These mechanisms lead to an increase in reactive oxygen species (ROS) formation and peroxidation of the lipids from cell membrane, i.e. oxidative stress, which results in diverse stress proteins[10] up-regulated, membrane damage and leakage of cellular components. As a consequence of this toxicity against bacterial cell, the bacterial growth is inhibited. [1, 32]. Silver ions have antibacterial activity themselves, therefore their release might also be involved in the antibacterial activity. However, they have demonstrated little antimicrobial action due to pronounced *in vivo* reaction with ions that compose body fluids thus precluding their activity, reason why the stability of phyto-MNPs is regarded as a crucial parameter [5, 34, 48]. Another proposed antimicrobial mechanism is based on the (iii) binding of Ag ions and phyto-AgNPs with proteins that contains sulfur residue (i.e. both intracellular and membrane proteins) and with phosphate groups of macromolecules like the deoxyribonucleic acid (DNA). These actions will disturb respiration functions, replication machinery and membrane permeability of cell, altogether leading to intracellular content's leakage, production of ROS, plasmid and chromosomal DNA damage and culminating in cell death [1, 5, 23, 35, 43]. A further possible mechanism is the (iv) electrostatic interaction of Ag ions and phyto-AgNPs (positively charged) with the membrane (negatively charged), leading to proton motive force dissipation and structural changes, resulting in the development of pore-induced intracellular traps (PITs) in the bacterial cell wall, where Ag ions and phyto-AgNPs accumulate, thereby resulting in enhanced membrane permeability, which culminates in cell death [5, 23, 35]. Other possibility is the (vi) interaction between the bacterial cell wall and phyto-AgNPs, that might destabilize the potential of plasma membrane and reduce the intracellular levels of adenosine triphosphate (ATP), resulting in death of bacterial cell [32]. The antibacterial properties can also come from (v) penetrating/anchoring to cell wall of the bacteria followed by modulation

[6] Phenolic compounds, flavonoids, saponins, carbohydrates, terpenoids and tannins.
[7] Key cofactor in the amino acids synthesis.
[8] An enzyme bearing antioxidant activity.
[9] Glutathione plays a key role at protecting both the cell against oxidative stress and the thiol groups of proteins.
[10] Including bacterial heat shock proteins.

(desphosphorylation) of phosphotyrosine profile of putative key bacterial peptides[11], disturbing protein functions and synthesis, resulting in cellular signaling modulation and bacterial growth inhibition [13, 23, 43]. On the other hand, the anti-biofilm activity is based on the inhibition of exopolysaccharide secretion, key in the adhesion of microorganisms and, consequently, in production of biofilm[12] [6].

Diverse phyto-AgNPs characteristics affect the antibacterial action, such as size [5, 17, 19, 31, 43], morphology [4, 11, 17, 19], crystallographic structure [4], agglomeration state [17, 43], stability [5, 26], surface roughness [5], chemistry of the surface and charge [43], large surface area/volume ratio [4, 12-14]; together with external factors, like the starting bacterial number and the bacterial strain [11-13, 31]. The antimicrobial activity is also concentration dependent (an increase in concentration leads to higher activity) [4, 6, 10, 12, 13, 31]. The ultrafine structure seems to be involved in the antibacterial action [4]; additionally, both hexagonal and spherical shapes appear to be responsible for the activity against Gram-negative bacteria [4]. Regarding the size, phyto-MNPs with size under 10 nm are able to adhere to the cell membrane of bacteria, penetrate and diffuse, leading to cell death through growth inhibition. This result indicates that by decreasing the particle size, bactericidal activity can be increased [5, 17, 31, 35, 43]. The interaction that occurs during phyto-MNPs formation between metal ions and plant metabolites, i.e. the electrostatic attractions among positively/neutrally charged nanoparticles and negatively charged phytocomponents, act synergistically in order to enhance biological activity of plants [11, 19]. An illustrative example are the PEG-coated AgNPs, formulated by Rolim *et al.* [43], in which PEG increased the phyto-AgNPs hydrodynamic size (<50 nm) and created an additional layer on AgNPs surface, interfering with antimicrobial activity. This way, uncoated phyto-AgNPs presented themselves smaller (<40 nm), with phytochemicals attached to the surface, that can enhance antibacterial activity, thus leading to better antibacterial activity than PEG-AgNPs. Appapalam and Panchamoorthy [5] showed that an increased surface roughness might reduce the biofilm formation/bacterial colonization, thus increasing overall bactericidal effect. Furthermore, the antibacterial activity is higher in Gram-negative bacteria, since they have a slim peptidoglycan layer, which has a lipopolysaccharide layer enveloping, resulting in lack of rigidity and strength, thus facilitating phyto-AgNPs penetration into cells; by contrast, Gram-positive bacteria possesses a cell wall with a rigid and thick peptidoglycan layer, complicating the entrance of phyto-AgNPs to the cell [11, 13, 35].

[11] Key for the progression of cell cycle and for capsular polysaccharides' synthesis.
[12] Biofilm protects microorganisms against hostile environmental factors.

3.1.2 Antioxidant activity

Concerning the antioxidative potential, phyto-AgNPs present free radical scavenging activity, and both reductive and anti-lipid peroxidation properties [5, 11, 12, 19, 22, 23, 31, 34, 46, 48]. The antioxidant activity of phyto-AgNPs can be attributed to the presence of phytoconstituents[13] on the phyto-MNPs surface, with a variety of functional groups [1, 4, 5, 11, 19, 23, 35, 43, 46, 48]. The large surface area/volume ratio, together with an electrostatic field composed by antioxidant phytoconstituents on the phyto-MNPs surface give them a high propensity to interact and reduce species like DPPH [23].

The antioxidant ability of phyto-AgNPs is concentration-dependent (higher concentration, higher activity) [1, 12, 22, 23] and the phyto-AgNPs volume directly interferes with the anti-lipid peroxidation (higher volume, higher activity) [23]. The increasing concentration of plant extract also seems to increase the scavenging activity [1]. Just like in the antimicrobial activity, the interaction between metal ions and plant metabolites enhance the activity of plants [11, 19, 23].

Phyto-AgNPs appear to have a positive effect on anti-aging enzymes, showing anti-elastase, anti-collagenase and anti-tyrosinase activity [19, 31]. The anti-aging activity of phyto-AgNPs is correlated with the total flavonoid and phenolic contents [31]. Together, the antioxidant and anti-aging activities of phyto-AgNPs open doors for their utilization in cosmetics [19, 31].

3.1.3 Anticancer activity

Phyto-AgNPs can be used also as anticancer agents and/or drug delivery carriers aimed at oncological applications [1, 3, 4, 12, 26, 32, 35], for both diagnosis and therapy [6]. One of the most relevant cytotoxicity mechanisms in cancerous lines is the activation of the apoptosis or necrosis processes [3, 4]. An explanation for the induced apoptosis may be the (i) arrest of sub-G1 phase and, consequently, the decreased cellular population of G0/G1 phase [32]. Phyto-AgNPs can also (ii) damage the DNA by affecting the respiratory chain of mitochondria, that are directly correlated with ATP production [32]. Other motive is the (iii) ROS[14] formation, which influence pathways of signal transduction that activate apoptosis, and is associated with the potential of mitochondrial transmembrane and rupture of respiration. Consequently, it can be stated that an increase in the cellular ROS results in cellular damage [32]. The initial production of ROS by phyto-AgNPs generates Ag ions, in acidic conditions,

[13] Phenolic compounds, flavonoids, saponins, carbohydrates, terpenoids and tannins.
[14] Especially O_2^- and H_2O_2.

through a process of oxidative dissolution[15][32]. The (iv) silver ions released from phyto-AgNPs also explain the toxicity effect against cancer cells [32]. In a western blot analysis of a lysate of mouse melanoma cell line (B16) treated with phyto-AgNPs carried out by Mukherjee *et al.* [32], it was observed expression of active caspase-3 and up-regulated p53 protein, that also lead to apoptosis. Moreover, an antimelanoma mechanism is the (v) inhibition of tyrosinase activity. This inhibitory effect may derivate from phytochemicals, like flavonoids, absorbed on the phyto-MNPs surface, since they have an intrinsic capability to chelate two copper atoms at the tyrosinase active site. Besides de antimelanoma potential, the inhibition of tyrosinase activity provides to the phyto-AgNPs capabilities for cosmetic applications [19].

The anticancer activity of phyto-AgNPs relays on the stability and characteristics of the MNPs, including its phytocompounds and nutrients concentration [26, 46]. Higher concentration of secondary metabolites and nutrients boosts cytotoxicity [46]. Additionally, the cytotoxicity of phyto-AgNPs is concentration dependent [4, 32, 46], and also relays on external factors such as pH of the environment [32]. Since the silver ions are responsible for death of cancer cells, their concentration directly affects the anticancer activity, that varies from cancer cells to normal cells, explaining the selective toxicity towards the former. This variation may be explained by the slightly acidic pH of cancer cells compared to normal cells; the release of Ag ions is pH dependent (at lower pH more ions are released) and, given that silver ions promote formation of ROS, ROS concentration is also considered pH-dependent [32].

3.1.4 Wound healing activity

Phyto-AgNPs are also attributed anti-inflammatory and anti-angiogenesis properties, which contribute to their utilization as wound healing agents [1, 6, 14]. This way, phyto-AgNPs and silver-based ointments have a role as wound healing agents for topical application, without scar formation [1, 5, 6, 8, 14, 34]. A great example is a study conducted by Arya *et al.* [6], in which a formulation of phyto-AgNPs and Carbopol showed remarkable wound healing. Naraginti *et al.* [34] conducted a study with phyto-Au and AgNPs, with the aim of assess and compare their wound healing activities. After topical application, both phyto-MNPs accelerated the wound healing rate, leading to complete epithelialization after 14 days. This study allowed to understand that the wound healing activity is the result of various processes. One of them is the (i) increase in migration and proliferation of fibroblasts and the increased

[15] $2Ag + H_2O_2 + 2H^+ \rightarrow 2Ag^+ + 2H_2O$.

generation of connective tissue protein. Additionally, it can be verified a (ii) modulation of collagen and extracellular matrix (ECM) during regeneration and repair process and an increase in formation of granulation tissue. Furthermore, in the granulation tissue, the (iii) inflammatory cells decrease and the active fibroblasts and collagen deposition increase, leading to the acceleration of epidermal differentiation and, finally, the (iv) production of a dense matrix of collagen, organized, without lengthened inflammatory response [34]. Another mechanism underlying the wound healing potential of phyto-AgNPs is (v) the immunomodulation, in particular cytokine modulation, decreasing inflammation and scar formation and leading to quick wound epithelialization, contraction and closure [6, 8, 14]. Also, the (vi) anti-inflammatory activity of phyto-AgNPs itself also promotes wound healing [1].

In addition to the abovementioned critical factors, shape, structure and size of MNPs should always be controlled, no matter the pretended activity, given their key technological importance and correlation with phyto-MNPs characteristics [19, 38]. For example, the spherical shape is a necessary feature for biological use [11].

3.2 Phyto gold nanoparticles

Gold has an inert nature, possesses low toxicity and has been utilized as a medicine [51]. It also possess great bactericidal and bacteriostatic properties [14].

Phyto-AuNPs exhibit unique electronic and optic properties, self-assembled nature, biostability, low cytotoxicity, and enhanced drug delivery features, which potentiate their use in the biomedicine field [7, 15, 19, 27, 34, 37, 38, 40, 51]. Phyto-AuNPs can penetrate the SC, which make them of great interest in plastic surgery [27]. The drug delivery property makes phyto-AuNPs ideal as carriers for both large biomolecules and smaller drug molecules, in targeted therapies such as anticancer therapy, for which preclinical and clinical trials of AuNPs-based drugs are already ongoing [7, 19, 27, 37, 51]. As already stated, phyto-AuNPs possess cytotoxic effect against cancer cell lines, such as melanoma cells, even at low concentrations, making them a drug of interest in treatment of skin cancer [7, 18-20, 37, 51]. Additionally, other biological activities should be mentioned, namely antioxidant, anti-aging, moisture retention [7, 11, 15, 18-20], sun protection [15], antibacterial and antifungal [7, 11, 19, 40], anti-inflammatory and wound healing [14, 34, 40]. Furthermore, phyto-AuNPs also show potential for photothermal therapy and application in sensing, imaging and labelling [7, 11, 34, 38, 40].

Along with their biological activities, phyto-AuNPs stand as a perfect model to study the skin penetration of phyto-MNPs. For this reason, Lin *et al.* [27] used human skin to conduct a penetration study, with the aiming at understanding the elemental interaction between phyto-AuNPs and skin, toward their use in plastic surgery and the development of an unprecedented transdermal transporter. This study focused on impacting factors of phyto-AuNPs regarding skin penetration and permeation, like size, physical state, physiochemical parameters and surface polarity, together with the influence of vehicles (its nature and surface polarity). The penetration was greater in the SC and outer layer of skin, showing lower values in the inner layer. The phyto-AuNPs synthesized with the highest concentration of plant extract entered the inner layers of skin after 24h of exposition, but the penetration was heterogeneous. Contrarily, hydrophilic phyto-AuNPs stabilized using citrate with 15 nm diameter evidenced no penetration in inner layers, however a hydrophobic surface can overcome this difficulty. This way, phyto-MNP with a hydrophobic surface seems to be more promising for skin penetration. The size range of phyto-AuNPs[16] in the Lin *et al.* [27] study shows a similar penetration pathway to that observed with drug molecules; in opposition, their particulate nature decreases the speed of diffusion via intercellular direction, essentially dominated by pores belonging to lipidic fluid. These results demonstrate the size of phyto-MNPs as a key factor for permeation of skin. Hydrophilic phyto-AuNPs of 15 nm diameter stabilized with citrate aggregated after skin exposure for 24h, leading to lower accessibility of individually dispersed phyto-AuNPs with higher probability of penetrating SC. Contrarily, other dispersions of phyto-AuNPs were physically stable in the beginning and after 24h of *in vitro* skin exposure.

3.2.1 Cosmetic applications

Phyto-AuNPs anti-aging, anti-inflammatory, moisture retention, anti-tyrosinase and antioxidant properties have attracted attention concerning its use in cosmetics [7, 15, 18-20]. Jimenez-Perez *et al.* [18] assessed the moisture retention activity of phyto-AuNPs, concluding that it was nearly similar to the one achieved with glycerin. Moreover, one of the most interesting features of phyto-AuNPs is their capability of enhancing the sun protection factor (SPF) and thus to be used as an effective skin protector against UV light, or as a booster, in sunscreen formulations, which is only possible thanks to their considerable photostability [15].

[16] 10 to 140 nm.

The antioxidant activity of phyto-AuNPs can be evaluated recurring to different assessments [15, 18, 20]: (i) protection against fibroblasts injured by ROS due to its free radical scavenging activity [11, 18, 20] and promotion of cell recovery [20]; and (ii) prevention of the oxidative degradation of biological molecules, proved by the use of model biomolecules such as the protein cytochrome C (Cyt-c) and the nucleoside 4-thiothymidine (S^4TdR), even in absence of H_2O_2 and at low concentrations, thus preventing ROS production induced by UV[17] [15]. Despite ascorbic acid is a known powerful antioxidant, phyto-AuNPs present a better option owing to their cell recovering properties [20]. The antioxidant activity of phyto-AuNPs can be increased by the interaction between metallic ions and phytochemicals during phyto-MNPs formation, through electrostatic interactions among phytochemicals[18] (negatively charged) and phyto-AuNPs (positively/neutrally charged) [11, 15, 18, 20]. Another factor that impacts the antioxidant activity[19] of phyto-AuNPs is the dose [15, 18, 20]; indeed, increasing the dose increases the cell viability and recovery, and decreases the Cyt-c degradation, the oxidation and the degradation of S^4TdR [15, 18, 20].

The utilization of phyto-AuNPs as a booster or principal ingredient in sunscreen formulations for enhancing SPF is based on two mechanisms: their ability to absorb in the UV region (between 290 and 400 nm), filtering the UV light (both UVB and UVA) and functioning as a chemical filter; and the antioxidant activity, due to a phenolic layer in the phyto-AuNPs surface, that prevents UV light-induced ROS production, acting as a biological filter [15]. Since phyto-AuNPs represent both chemical and biological filters against sun irradiation, they decrease the need for physical filters in sunscreen formulations, which is a big advantage given the fact that these leave a white residue on skin [15]. Filtration of UV light, and consequently SPF value, is dose-dependent, increasing with a higher concentration of phyto-AuNPs [15].

3.2.2 Antimicrobial activity

Phyto-AuNPs show antifungal [7, 40] and antibacterial activity, overcoming the antibiotics resistance [7, 11, 34, 40]. The antibacterial activity is highlighted by disrupting and reducing the biofilm [40], while the antifungal activity relays in the H^+-ATPase inhibition [40]. The antibacterial activity depends on the different bacterial cell walls and on the surface properties of phyto-MNPs [7, 11, 40]. Surface properties are affected by the biomolecules attached and directly interfere with the process of adhesion and penetration into the bacterial

[17] TdR and R-SOH.
[18] Polyphenols, polysaccharides, terpenoids and flavonoids.
[19] e.g. protection against ROS' cell damage, cell recovery, degradation of Cyt-c and S4TdR.

cell wall, by electrostatic interactions [7, 40]. The variation on the bacterial cell wall compounds explains the higher activity on Gram-negative bacteria [11].

The antimicrobial action open doors for the topical use of phyto-AuNPs, for dermatological disorders, skin disinfectants and wound healing formulations [7, 18, 20, 40], while the antioxidant and anti-inflammatory properties could reduce side effects associated with dermatological infections [7].

3.2.3 Wound healing activity

Phyto-AuNPs also possess wound healing activity and prevents formation of scars [34, 40], which makes them a good skin agent for recovery of damaged cells [20]. In a study conducted by Raghuwanshi *et al.* [40], a 1% ointment gel (phyto-AuNP-Carbopol®934) was formulated. This gel showed remarkable wound healing properties and presented the viscosity and spreadability needed for topical applications. Its wound ability was seen by (i) increasing wound contraction, (ii) decreasing epithelization period, (iii) increasing hydroxyproline content, (iv) increasing tensile strength, (v) regulating collagen deposition, (vi) organizing extracellular matrix and (vii) generating blood vessels. This ointment leaded to fast healing and wound closure through (viii) formation of granular tissue, (ix) quick gathering of collagen fibrils, and (x) rejuvenation of epithelium. Wound healing activity is concentration-dependent and highly dependent on appropriate spreadability of phyto-MNPs for easily deliver the active molecules [40]. An example is the tensile strength, that increases with increasing concentration [40].

3.2.4 Anticancer activity

Phyto-AuNPs possess anticancer activity against melanoma cells [7, 51], which opens doors for their use as therapeutic agents against skin cancers [7, 34, 37, 51]. In a study leaded by Wu *et al.* [51], this activity was shown by the induction of apoptosis on melanoma cells. This phenomenon was demonstrated by decrease in viable cancer cells (with a minimal cytotoxic concentration for 50% reduction - 10 μg/mL), ROS generation, reduced potential and increased permeability of the mitochondrial membrane, increased apoptotic cells (early and late), increased expression of apoptotic genes and decreased antiapoptotic gene expression. The anticancer activity of phyto-AuNPs also relays on anti-tyrosinase potential, downregulated melanogenesis genes and reduction in the melanin content in B16 cells and, consequently, the whitening effect [18-20].

The key mechanism responsible for the anticancer activity of phyto-AuNPs is the apoptosis, whose principal biochemical changes are caspases' activation, reduction in the permeability of cellular membrane and disintegration of DNA [51]. Different cytotoxic signals such as death receptors, growth deprivation and ROS trigger apoptotic signaling, resulted in the scavenge of damaged DNA, leading to carcinogenesis [51]. ROS activity is reflected in decreased potential and increased permeability of mitochondrial membrane. The damage in the mitochondrial membrane leads to proapoptotic proteins leakage from the cytoplasm (like cytochrome C), that in turn activates apoptotic proteins (such as caspases 9 and 3). Caspases cleave cytoskeletal and nuclear proteins through activation of enzymes that degrade DNA; furthermore, caspase 3 complexes with cytochrome C, caspase 9 and Apaf1 to form an apoptosome, leading to apoptosis [51]. In a polymerase chain reaction (PCR) conducted by Wu *et al.* [51], it was observed up-regulation of caspase 3 and 9, bcl-2 associated X (Bax) and Bid (apoptotic genes) and down-regulation of antiapoptotic Bcl2 gene, so it can be stated that phyto-AuNPs mimics BH3, binding to the Bcl2[20] group hydrophobic groove, thereby inducing apoptosis.

Like formerly said in the phyto-AgNPs topic, the anti-tyrosinase activity may be attributed to phytochemicals, like flavonoids, absorbed on the phyto-MNPs surface, since they have a capacity to chelate two copper at the tyrosinase's active site [18-20]; other phytochemicals, e.g. coumaric acid, competitively inhibits DOPA[21], thanks to its structural similarity with tyrosinase [18]. Another finding is the transcriptional tyrosinase inhibition, demonstrated by the downregulation of the melanogenesis-associated transcription factor (MITF), that regulates tyrosinase, and tyrosinase gene (responsible for synthesizing the tyrosinase) [18]. The antimelanogenic activity of phyto-AuNPs in B16 cells may be due to presence of polyphenols and aromatic acids (such as syringaesinol and coumaric acid), that are major tyrosinase inhibitors, and reduce the accumulation of pigments related to age in human dermal fibroblast (HDF). Additionally, other bioactive compounds (phenolic compounds, ginsenosides, among others) also reduce melanin synthesis and prevent increase in intracellular levels of ROS, responsible for changing the oxidation-reduction state of cellular membrane protein, thus raising melanin production, which translates in darker skin [18, 20]. The tyrosinase inhibition and, subsequently, the antimelanogenic effect suggest phyto-AuNPs involvement in the whitening of the skin [18, 20]. Phyto-AuNPs undergo uptake by normal and

[20] Bcl2 is a family of proteins with a BH domain, that include Bcl2, Bid and Bax genes, that regulates the apoptotic pathway mediated by mitochondria.
[21] A subtract and cofactor utilized by human tyrosinase.

cancer cells via endocytosis via a clathrin-mediated pathway [18]. However, genotoxic stress particularly affects melanoma cells and, since phyto-AuNPs provoke nucleus damage, melanoma cells are highly sensitive to phyto-AuNPs [7]. Phyto-AuNPs can even be used for photothermal treatment of cancer cells since, using light to irradiate, they trigger native heating [27]. These findings support the role of phyto-AuNPs as antimelanogenic agents.

Diverse factors influence the anticancer activity of phyto-AuNPs, among them size [7, 37, 51], dose [7, 18, 20, 37, 51] and the phytochemicals present in the phyto-AuNPs [7, 18, 20], and the type of cancer [37]. Secondary metabolites, in particular polyphenols, seem to stimulate the anticancer activity [7]. Small size, for its part, enhances the drug delivery ability and cell penetration [51]; in fact, there is an optimum size that leads to maximum density of the attached biocompounds, which changes their configuration. This change leads to altered communication between cancer and normal cells, culminating in inhibition of tumor growth [7]. Higher dose leads to an increase in expression of apoptotic gene [51], a higher suppression of tyrosinase activity and a reduction in production of melanin. Consequently, there is a decrease in the content of melanin [18, 20] and in the tyrosinase gene expression [18], and a reduction in the number of viable B16 melanoma cells [18].

3.3 Phyto zinc and zinc oxide nanoparticles

Zinc and zinc oxide are inorganic materials that possess various advantages over organic materials due to their intrinsic properties, in particular their (photo)stability, the capability of withstanding harsh processes and/or high temperature treatments, and a broad range of radiation absorption. Furthermore, they are considered as generally recognized as safe (GRAS) components for humans.

Consequently, phyto-ZnNPs and ZnONPs have been applied in diverse fields, in particular as protective agents against UV rays, since they have the ability to absorb UV radiations and, therefore, to be incorporated in ointments, lotions and cosmetic formulations. Moreover, these phyto-MNPs also find applications as antioxidants in cosmetics that penetrates the SC of skin, protects against ROS and improves cellular healing; as preservatives to substitute the chemical options or even as biocidals acting synergistically with organic agents, given that the ions present antimicrobial nature; and, finally, as moisturizers [30, 44, 47, 48]. Due to their enhanced biological activities[22], phyto-ZnNPs and ZnONPs have gained a lot of interest in biomedicine, drug delivery, cosmetic and dermopharmaceutical applications.

[22] Antifungal and antibacterial against wide spectrum microbes (even in reduced quantities), antioxidant, wound healing, anticancer and absorption of UV radiations.

Consequently, phyto-ZnNPs and ZnONPs are auspicious nanomaterials, with large surface area and lasting shelf life [2, 21, 28, 30, 44, 48]. The efficacy of these nanoparticles mostly relies on the metallic concentration, on the size and on the level of polydispersity of phyto-MNPs [2, 28, 44, 47].

3.3.1 Antimicrobial activity

Phyto-ZnNPs and ZnONPs are effective bactericidal, fungicidal, bacteriostatic and fungistatic agents, even at low concentrations, reason why they have been widely employed as antimicrobial agents in creams, lotions and ointments, to treat skin infections. [44].

The fungal defense mechanism against phyto-ZnONPs leads to an increase in carbohydrates and nucleic acid levels, whose accumulation leads to the deformed fungal hyphae; this, together with the prevention of developing conidiophores, culminate in cell death of fungi [44]. The antibacterial activity of these phyto-MNPs is demonstrated in various ways, such as morphological changes in bacteria and in biofilm, inhibition of biofilm synthesis, biofilm roughness enhancement and diminishment in biofilm density [2]. The bacterial morphological changes caused by these phyto-MNPs happen in five steps: interaction with membrane, integration with membrane, blebs on the membrane, membrane clumping and damage. [2]. Bacteriostatic/fungistatic activities of these phyto-MNPs may be a result of the (i) attached phytochemicals (in specific alkaloids, phenol and flavonoids) since they are effective microbiocidals [2, 44]. Another proposed mechanism for the antibacterial potential is the (ii) inhibition of bacterial enzymes, for example glutathione reductases, thiol peroxidases and dehydrogenases. Oxygen of the phyto-MNPs reacts with proteins of bacterial cell membrane having thiol functionalities (sulfhydryl groups, -SH), to erase hydrogen atoms in form of water molecules, developing a R-S-S-R bond together with S atoms, thereby blocking bacterial respiration, which culminated in inactivation of DNA replication and protein synthesis, altered membrane permeability and cell lysis [44, 47]. Other reason for the antibacterial activity, including the reduced biofilm formation, is the (iii) generation of ROS[23]. ROS resulted in lipid peroxidation of bacterial cell membrane and inhibition of cell growth [2, 44]. The antibacterial potential also relays on (iv) blockage of cellular processes and cellular immobilization of bacteria, by sticking with its surface. Phyto-ZnONPs penetrate both outer and inner cell wall of bacteria, and obstruct transport channels, which results in intracellular leakage, disrupted nuclear functions, ineffectiveness and cleavage of bacterial cell membrane, culminating in cell

[23] Specifically, superoxide radical (O_2^-), peroxide anions (O_2^{2-}), hydroxide (OH^-), and hydrogen peroxide (H_2O_2).

death [44, 47]. A distinct antibacterial mechanism of phyto-ZnONPs is based on the (v) lethal effect of electrostatic attachment between ZnO (charged positively) and cell wall of bacteria (charged negatively), ending in released Zn^{2+} ions in the cytoplasm. The ions released and the nanoparticles itself bind the bacterial DNA molecule with nucleic acids strands, leading to their cross-linkage. This phenomenon culminates in a DNA molecule with disrupted helical structure, which causes biochemical processes (such as protein denaturation) and, finally, complete cellular destruction of bacteria [2, 21, 48]. The (vi) increased roughness of biofilm may be due to macromolecules denaturation[24] and phyto-MNPs coagulation in biofilm. Additionally, antibacterial activity also happens via (vii) disruption of motility, by means of lowering pyocyanin concentration, as it was proved with *P. aeruginosa* [2]. Furthermore, another antibacterial mechanism is the (viii) activation of electrons [48].

In all the studies, the antimicrobial activity of phyto-ZnNPs and ZnONPs was concentration-dependent, including the reduction of both biofilm formation and swarming motility [2, 28, 44]. However, antimicrobial activities of these phyto-MNPs are dependent on several parameters, involving the biochemical, physiological and morphological differences between Gram-positive and negative strains, temperature during the synthesis, physiological characteristics and specific properties of phyto-ZnNPs and ZnONPs (such as nanometric size and large area of the surface to volume), level of contact between phyto-MNPs and bacteria and modification of surface with chemical/biological substances [2, 21, 28, 44, 47, 48]. To illustrate what was said above, smaller phyto-MNPs can enter inside the cell, causing toxicity effects, and the higher ratio surface area/volume gives phyto-MNPs adsorption, absorption and penetration capabilities on the bacterial cell surface [2, 28, 44, 47, 48]. In studies performed by Alavi *et al.* [2], Mahdavi *et al.* [28] and S *et al.* [44], phyto-ZnNPs and ZnONPs showed higher antibacterial effect on Gram-positive bacteria, which may be explained by the growth rate differences, the neutralization of the surface potential and the properties of cell wall/bacterial stability. Gram-negative bacteria wall is made of 90% lipids and two cellular membranes (inner and outer membrane); the external membrane has lipopolysaccharide structures and the inner membrane has a thin layer (≈ 8 nm) of peptidoglycan, with lipoprotein structures. By contrast, Gram-positive bacteria lack external membrane (lack lipopolysaccharide structures) and have a thick peptidoglycan layer (≈ 80 nm) rich in teichoic acids, which are sensitive to phyto-ZnONPs. It was also observed, in a study commanded by Alavi *et al.* [2], an inverse relationship between time of exposure and bacterial growth. At

[24] Proteins, nucleic acids and polysaccharides.

lower concentrations of phyto-ZnONPs, there was a delay in the bacterial growth (bacteriostatic effect) but, for higher concentrations, complete inhibition was observed (bactericidal effect). In a study conducted by Sumbal *et al.* [48], monometallic (Zn) and bimetallic (Ag and Zn) phyto-NPs were synthesized. Among them, bimetallic phyto-NPs showed better antibacterial activity and proved to be capable of circumvent the immune effects (both passive and side) commonly seen with biocidal medications. The higher antibacterial activity of bimetallic phyto-NPs may be due to the synergism achieved by using two metals and higher reactivity, resulting from dissimilar intensity of strength, binding, configuration, and interaction.

3.3.2 Antioxidant activity

Several studies demonstrated the antioxidant activity of phyto-ZnNPs and ZnONPs [28, 44, 48, 49], that proved to be efficient in quenching free radicals [44]. The antioxidant activity appears to be concentration-dependent, including the DPPH free radical scavenging activity and the linoleic inhibition activity [2, 21, 44]. In the Sumbal *et al.* [48] study, the antioxidant activity of bimetallic phyto-MNPs varied along with different metallic concentrations, thus demonstrating the role of the content of phyto-MNPs. Metabolic processes or external factors with potential to provoke skin damage generate free radicals[25], acting as acceptors for hydrogen ions, leading to oxidized free radicals [44]; therefore, the antioxidant activity relays on the secondary metabolites with free hydroxyl groups present on the phyto-MNPs surface[26] that possess the ability of donating hydrogen [2, 44, 48]. Another mechanism that might be involved in the antioxidant activity is the release of zinc ions from the phyto-MNPs [48].

The studies conducted by Mahdavi *et al.* [28] and S *et al.* [44] analyzed the antioxidant effect of an ointment formulated with phyto-ZnNPs and ZnONPs. Pus and inflammation in the wound are caused by the build-up of free radicals, that leads to an excess of inflammatory cells[27]; the antioxidant compounds present in the ointment destroy free radicals, lighten the shearing and prevent pus production, thus accelerating wound healing. Additionally, these ointments exhibited great stability and moisturizing properties, with no skin irritation, turning these nano-based formulations into a great option to alleviate infections of human skin and cellular damage induced by oxidative stress.

[25] Such as DPPH, a free radical stable, lipophilic and nitrogen-centered.
[26] Including flavones, flavanols, flavonoids and flavanones.
[27] Lymphocytes, macrophages and neutrophils.

Khan *et al.* [21] analyzed the antioxidant and antibacterial activity of phyto-Cu-doped ZnONPs, produced by two different methods, that will be further referred as MNPs1 and MNPs2, and phyto-ZnONPs. Both doped MNPs showed higher DPPH free radical scavenging activity than un-doped MNPs. MNPs2 showed greater DPPH free radical scavenging activity than butylated hydroxytoluene (BHT)[28] and presented the lowest oxidation of linoleic acid. Concluding, MNPs2 had the best antioxidant activity, while MNPs1 activity was comparable to that of BHT, but higher than ZnONPs. All the MNPs had great antimicrobial activity, and doped MNPs exhibited great activity against fungi; nevertheless, MNPs2 showed greater antibacterial and antifungal activities. Just like zinc, copper ions also permeate and destroy the cellular membrane of bacteria, which explains the superior antibacterial activity of doped-MNPs [21]. The better efficacy of MNPs2 is intimately related with doping. When a transition metal is doped, there is an increment in the crystalline size, enhancing the surface area and decreasing particles size; these characteristics enhance the antioxidant and antibacterial activity. The described mechanism is best noticeable in MNPs2 than MNPs1, given that the doping used to synthesize MNPs by method 2 have a higher level of copper [21].

3.3.3 Wound healing activity

Phyto-Zn and ZnONPs even show a potential to be used as wound healing agents, as shown in the studies performed by Mahdavi *et al.* [28] and Shao *et al.* [47], in which the ointments formulated using phyto-ZnNPs exhibited excellent wound healing properties. Furthermore, comparing to standard treatments, these ointments achieved similar action and avoided the wound associated chronicity, reasons why these phyto-MNPs are gaining such importance in pharmaceutical and medicals fields, for wound healing purposes. The wound healing activity was demonstrated by several ways. There was an increase in the concentration of hexuronic acid, hexosamine and hydroxyproline, and also in the number of fibroblasts, fibrocyte and blood vessels, which leaded to higher ratio between fibroblasts and fibrocytes. Additionally, there was a reduction in the wound area, the number of lymphocytes and total cells. Consequently, the wound infection and the frequency of changing the dressings decreased, leading to lower costings and associated pain, which clearly are great advantages associated with the use of phyto-ZnNPs as wound healing agents.

Furthermore, as aforementioned, the antioxidant property eases cellular damage induced by oxidative stress, thus accelerating wound healing [28, 44].

[28] Standard antioxidant.

3.3.4 Cosmetic applications

Nowadays, phyto-ZnONPs are being widely used in lotions, ointments and creams, as a skin protector against cellular damages and sunburn provoked by UV rays. Thus, phyto-ZnONPs are added as ingredients in sunscreens formulations, blocking both UVA and UVB rays and increasing the sun protection factor (SPF). In terms of cosmetic appealing in the skin application, phyto-ZnONPs do not reflect light, so they appear transparent, unlike the traditional sunscreens with larger ZnO particles incorporated, that results in white milky appearance. In terms of efficacy, these nanocreams show even better absorption and UV filtration properties than standard creams [44].

3.4 Phyto copper and copper oxide nanoparticles

The promising activities of noble metals make them of great interest, however they cost can become an obstacle. Compared to the noble metals, such as Ag, Au or Pt, the transition metal copper is cheaper. Furthermore, Cu and CuO has diversified applications in both the laboratory and industry level, namely as additive in antibacterial formulations and germicides, and in cancer treatment [9, 33]. Besides these activities, copper is also applied for doping MNPs, enhancing their properties [21].

Phyto-CuNPs and CuONPs are particularly interesting given its good thermal and chemical stability, low toxicity and cost, great catalytic reusability and ease of manipulation. Comparing to both microbial and organic agents, these phyto-MNPs present themselves as stable, robust and with long shelf life [42]. Along with the enhanced physico-chemical properties, phyto-CuNPs and CuONPs also exhibit important biological activities, such as antioxidant and anticancer activities, which justify the investment in developing formulations with these phyto-MNPs for topical applications [33, 42].

3.4.1 Antioxidant activity

In a study performed by Rehana *et al.* [42], various phyto-CuONPs were synthesized using different plant extracts. *In vitro* antioxidant assays proved the radical scavenging properties of these phyto-MNPs, much better than that of chemically synthesized MNPs, as a consequence of secondary metabolites present in the extracts. Moreover, the antioxidant activity has proven to be concentration-dependent. Phyto-CuONPs synthesized using *Tamarindus indica* L. presented the highest activity, presumably as a result of the strong impact of extract's added bioactive compounds, such as proteins, amino acids, carbohydrates, glycosides, flavonoids, saponins, phenolic compounds and tannins. Phyto-CuONPs synthesized

using *Hibiscus rosa-sinensis* L. also exhibited powerful scavenging activity, followed by phyto-CuONPs synthesized using *Azadirachta Indica* A.Juss., *Murraya koenigii* (L.) Spreng., and *Moringa oleífera Lam.*. The enhanced medicinal activities of *T. indica* and *H. rosa-sinensis* phyto-CuONPs are reflected in the comparable antioxidant activity to standard ascorbic acid and can be explained by existence of additional phytochemicals in the plant extracts utilized to their synthesis [42].

3.4.2 Anticancer activity

In a study carried by Mukhopadhyay *et al.* [33], CuNPs were synthesized via plant-based route. These phyto-MNPs displayed anticancer activity against human skin carcinoma cells (B16F10). The auspicious cytotoxic effect of phyto-CuNPs may be due to their cellular internalization and continuous leakage of bioactive components. The cytotoxicity in B16F10 melanoma cells is achieved through many mechanisms, among them (i) increasing the lactate dehydrogenase (LDH) leakage, the ROS production and, consequently, the ROS content, decreasing GSH level (which leads to the elevation of ROS levels, such as superoxide radicals and superoxide levels, ending up in injured cancerous cells and death). In addition, (ii) upregulation of apoptotic genes such as apoptosis-inducing factor (AIF), caspase9, caspase3 and Bax, and (iii) downregulation of gene Bcl-2 (anti-apoptotic) also play an important role for the anticancer activity of phyto-CuNPs. It appears that all the mechanisms associated with the cytotoxic activity in B16F10 cells are dose-dependent.

The same study conducted a proteomic analysis, which allows to understand the changes that occurs at the protein level, that showed 39 different spots; among these, 24 were upregulated (62%) and 15 were downregulated (38%). Among expressed proteins, 18% comprehend apoptosis, 15% cell cycle capture, 10% cell signaling and 3% oxidative stress. The authors also run a reverse transcription polymerase chain reaction (RT-PCR), which shown that Anxa 5[29] and Hsp 71 kD were upregulated and proliferating cell nuclear antigen (PCNA) downregulated [33]. Some cellular components responsible for suppression of cellular proliferation (reducing tumor formation) and invasion (reducing metastasis), for apoptosis and for immunogenesis were upregulated. Other components, involved in angiogenesis of cancer cells, in metastasis, in cell replication, in transcription (decreasing tumor growth) and in suppressing apoptosis were downregulated [33].

[29] Tumor marker.

In respect to gene regulation, it was found that Bax, a pro-apoptotic gene associated to tumor suppression mediated by p53 was upregulated, bcl-2 (an anti-apoptotic gene) was downregulated and caspase-independent AIF, that leads to cell death through DNA fragmentation and chromosome condensation, was upregulated. In addition to these mechanisms, Bax, caspase 9 and caspase 3 expression seems to contribute to activate mitochondrial pathway during apoptosis in melanoma cells. Hence, both caspase independent and dependent factors induce programmed cellular death [33].

The proteomic and the gene analyses lead to the conclusion that phyto-CuNPs are potent anticancer agents, whose activity relays on a synergism between various mechanisms, including the regulation of proteins/enzymes and genes, that culminate in cancerous cell death.

The *in vivo* efficacy was evaluated in two ways: by checking tumor volumes alongside the treatment and by histological staining of tissue sections that were collected 19 days after treatment. *In vivo* cytotoxicity results showed that phyto-CuNPs and CuONPs strongly inhibit the growth, and even lead to the cellular destruction of tumor, as seen by an outstanding necrosis that co-occurred with nuclei of larger size and minimum or insufficient cytoplasm [33]. Taken together, these results prove the efficacy of phyto-CuNPs and CuONPs as anticancer agents, which supports their use to treat skin cancers.

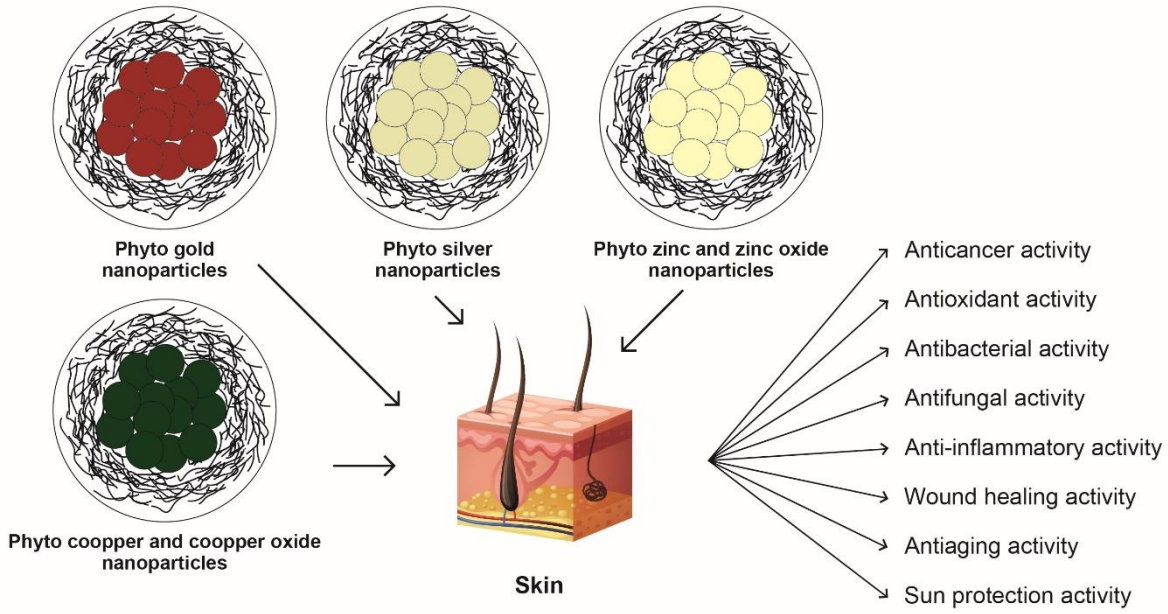


Figure 3 - Topical applications of phyto-metal-based nanoparticles.

4. Safety concerns / toxicity

In the last few years, there has been a growing concern about metal-based nanoparticles accumulation and toxicity on humans after long-term exposure, due to their increased use and interest for topical applications, resulting in an increase demand of cytotoxicity studies [32, 37, 51]. The majority of the recent studies focus on *in vitro* toxicity on only one cell-line, however there is the need to establish guidelines to confirm the results, with the aim of developing good models to ascertain the effect of phyto-MNPs in mammalian systems. This will provide the development of studies *in vivo* with established end points, which unlocks paths for clinical trials [7, 14, 26, 32, 51]. Besides the enormous advantages and the therapeutical and cosmetic potentials of phyto-MNP, their utility as a drug for dermopharmaceutical applications or as a cosmetic ingredient entirely relays on their safety profile [15, 32, 46].

Toxicity is highly influenced by structural, physical and chemical properties of nanoparticles, such as shape, size, level of agglomeration, charge and chemical composition of the surface [7, 32, 43]. The studies prove that cytotoxicity, and thus the cellular uptake, depends on the phyto-MNPs concentration and exposure time [7, 15, 19, 33, 43]. An example is the phytochemicals attached to the surface that, due to their intrinsic activities, reduce phyto-MNPs toxicity [28, 43]; other example is the size which, for phyto-MNPs uptake by cells, seems to be ideal around 50 nm [7]. Another important factor to consider is the possibility of existing contaminants from the manufacturing process, that can cause toxicity by themselves [34]. The uptake of gold and silver phyto-nanoparticles also seems to be dependent on an endoytosis pathway mediated by clathrin [19, 20].

Kummara *et al.* [26] conducted a thorough *in vitro* human and environmental toxicity study about phyto-AgNPs against HDF and brine shrimp. After incubation for 24h, HDF-adult (HDFa) cellular viability was not impacted by any phyto-AgNPs concentration (0 - 240 ppm) and, even at the highest concentration, there was not any remarkable change; however, chemically synthesized AgNPs were significant toxic at 120 - 240 ppm. The exact toxicity mechanism remains uncertain, but ROS production seems to be the cause of oxidative stress, that results in apoptosis. After 24h of treatment, HDFa cells treated with phyto-AgNPs showed no apoptotic features and no significant change in the intracellular ROS level, contrary to chemical AgNPs. Furthermore, phyto-AgNPs might inhibit ROS generation, scavenge free radicals and enhance antioxidative mechanisms in HDFa cells, producing a proactive effect. In conclusion, phyto-AgNPs did not show significant changes in ROS production, cell viability or apoptotic mechanisms in HDFa cells. Regarding environmental toxicity, phyto-AgNPs revealed

moderate mortality in brine shrimp, with concentration dependent lethality (240 ppm). However, chemicall AgNPs showed significant mortality in a lower concentration. Mukherjee *et al.* [32] leaded an *in vitro* study in human umbilical vein endothelial cells (HUVEC), during 24h of incubation, to prove the biocompability of phyto-AgNPs. In the range of concentrations used (3 - 30 μM) the plant-mediated green synthesized AgNPs had no toxic effect, while chemical AgNPs showed cytotoxicity even at low doses. Higher toxicity of chemical AgNPs seems to be caused by increased ROS production. Another study, leaded by Rolim *et al.* [43], evaluated the *in vitro* cytotoxicity of phyto-AgNPs and phyto-PEG-AgNPs on human epidermal keratinocytes (HaCaT) cells, to simulate their skin contact, during 24h and using a range of concentrations (0 - 150 μg/mL). Significant effect on cell viability was observed only at higher concentrations, nevertheless phyto-PEG-AgNPs needed more concentration (150 μg/mL) than AgNPs (50 μg/mL) to achieve cytotoxicity statistically significant, thus they can be considered less toxic. Naraginti *et al.* [34] examined, during 14 days, the *in vivo* effect of phyto-Au and AgNPs on mice wounds; the results showed that both nanoparticles did not adversely affect the animals. To determine the *in vitro* cytotoxicity of phyto-Au and AgNPs, Jimenez Perez *et al.* [19] exposed normal HDF cells to a range of concentrations (1 - 100 μg/mL) during 72h. Phyto-AuNPs showed no significant toxicity effect at any concentration; however, cells where more sensitive to phyto-AgNPs and the their viability decreased in a concentration dependent manner, as is the case with metal solutions.

Taken all together, these studies support the biocompatibility of phyto-AgNPs and their selective toxicity against cancerous cell lines, which make them ideal skin drug vehicles, at limited doses.

To analyze the *in vitro* cytotoxicity and the intracellular uptake of phyto-AuNPs, Gubitosa *et al.* [15] incubated, for 72h, human microvascular endothelial (HMVEC) and HDF cells with a range of concentrations (1.80×10^{-12} - 1.00×10^{-11} M), and determined the cell viability. The results showed no cytotoxicity between 1.80×10^{-12} - 3.60×10^{-12} M in any cell line, but cell viability decreased for the highest dose (1.00×10^{-11} M). Biological activities of the cell were not affected by phyto-AuNPs, as it was shown by HMVEC enriched with these nanoparticles that retained the capacity of differentiate in tube-like structures, forming an interconnecting network. Moreover, after the cells differentiate, phyto-AuNPs remained inside them. Concluding, in the concentration range of 1.80×10^{-12} - 3.60×10^{-12}, phyto-AuNPs are safe, but the highest dose is cytotoxic. To establish the safety of phyto-AuNPs, Benedec *et al.* [7] examined the cell viability of HDF cells, that mimics the mucosal and cutaneous route of these phyto-MNPs after topical application. For 24h of exposition, the *in vitro* level of toxicity

was inexistent up to 2.0 µg/mL of concentration and, for the highest (4.0 µg/mL), there was a small inhibition on cell viability. The dose-response curve shows an IC_{50} of 15.9±9.2 µg/mL. Thus, phyto-AuNPs are tolerated by HDF cells, which demonstrates their biocompatibility. In a study of Jimenez-Perez *et al.* [18], HDF cells were treated with various concentrations of phyto-AuNPs (1 - 100 µg/mL) and their *in vitro* viability was evaluated after 24, 48 and 72h. There was no morphological changes or loss of cell viability, thus proving the safety of these nanoparticles. Jimenez *et al.* [20] also evaluated the *in vitro* effect of phyto-AuNPs on cell viability and morphology of HDF cells, during 24h of incubation, using a wide range of concentrations (1 - 200 µg/mL). These nanoparticles did not show any adverse effect on morphology or cell viability, in any concentration, contrary to gold aqueous solution alone.

Regarding phyto-ZnNPs, Mahdavi *et al.* [28] incubated HUVEC cells during 24h with nanoparticles concentrations up to 1000 µg/mL. The results proved the inexistence of significant *in vitro* toxicity on account of the great viability of HUVEC cells. These results were supported by Shao *et al.* [47] *in vitro* study in cell lines of mouse fibroblast (L929), using various phyto-ZnO nano gel concentrations, for 24h of incubation. The lack of toxicity in fibroblasts cells, demonstrated by the excellent cell viability at all the concentrations tested, proved the biocompatibility of phyto-ZnONPs. These results justify the use of plant-mediated green synthesized zinc nanoparticles in dermopharmacy and cosmetics.

Rehana *et al.* [42] used normal human dermal fibroblast (NHDF) to study the *in vitro* cytotoxicity of phyto-CuONPs, at various concentrations (2 - 100 µg/mL), during 48h. The results proved the concentration-dependent cell toxicity, although no significant toxic effect was seen at any concentration (<20% toxic effect). Mukhopadhyay *et al.* [33] analyzed the *in vitro* cytotoxicity of phyto-CuNPs on normal fibroblasts (NiH3T3), at concentrations between 40 - 120 µg/mL, for 24h. Even at the highest concentration, >80% cells were viable, proving the minimum toxicity against normal cells. Furthermore, to assess *in vivo* toxicity derived from the usage of phyto-CuNPs to treat melanoma, a histological analysis to a portion of mouse liver demonstrated no noticeable signs of damage, proving their minimal secondary effects. The results of both studies prove the selective anticancer activity, giving support for the application of plant-mediated green synthesized copper nanoparticles.

5. Conclusions and future prospects

An empirical review of consumer consumption habits reveals a notorious preference in formulations with nature-based compounds. Thereby, in the last few years, there has been an exponential growing regarding the use of natural resources for developing safe and efficient formulations. Nowadays, nanotechnology is at the heart of technological development, considering that it overcomes most of the limitations of other technological areas. In order to embrace the synergism between nanotechnology and natural resources, green synthesis of nanoparticles has recently received considerable attention. Among all the potential natural starting materials, the diversity, availability, renovation, low cost and valuable phytoconstituents of plants turns them into the best option. The history tells us that metals have been used for long to treat various diseases, however their toxicity and low bioavailability have been the main constraints. Combining the advantages of plant-mediated green synthesis of nanoparticles and the therapeutical potentials of metals, phyto-MNPs have raised as valuable assets in pharmaceutical and cosmetic fields. Due to the broad range of applications, the present review focus on skin applications, particularly of Zn, Cu, Au and Ag phyto-nanoparticles, which have exhibited wide biological activities including anticancer, antioxidant, antimicrobial and wound healing activities.

In the near future, a lot of investigation needs to be done with the aim of understanding the fully potential activities of phyto-MNPs. In fact, some studies already investigated other possible biological activities. Sumbal *et al.* [48] explored the antileishmanial activity of phyto-ZnO and phyto-AgNPs while Mukherjee *et al.* [32] studied the fluorescence of phyto-AgNPs inside B16 cells, that unlocks paths for their use in fluorescence imaging for melanoma theranostic.

There are still major questions to be answered regarding the production of the plant extract and the synthesis of plant-mediated metal-based nanoparticles. The diversity of plants means that their full potential for the formation of nanoparticles is still unknown. Given the vast components of the plant extract, there is the need to proceed to their isolation and study their functionalities, with the aim of understanding the phytochemicals responsible for the reduction and the stabilization. The majority of the studies only analyses the extract as a whole, meaning that there is a lack of knowledge regarding the functions of each compound and, also, regarding the role of enzymes in the synthesis of phyto-MNPs. Future studies with isolated phytochemicals and enzymes will allow the synthesis of phyto-MNPs using only one or a few compounds, instead of the whole extract, facilitating the standardization. To achieve the technological reproducibility, the raw material needs to be standardized, which means that

following a general protocol, anyone will achieve the same results. In synthesis confirmation techniques, the color seems to have slightly differences according to the plant extract and its concentration; with the standardization, these variations will be less noticeable. The full control over the production process will allow the production of phyto-MNPs with desirable characteristics, in an industrial way, making them new dermopharmaceutical and cosmetic agents.

The reviewed studies utilized mostly microscopic techniques (e.g. TEM and SEM) to analyze size and shape of phyto-MNPs; nevertheless, DLS should be the first choice, because it determines the hydrodynamic size, and the microscopic techniques should serve only for confirmation. As a rule, the hydrodynamic size is bigger because it includes the hydration and the molecular layers (due to the phytochemicals attached to the surface of nanoparticles), which cannot be visualized in microscopic analyses.

Some of the analyzed studies showed big problems, for example the lack of comparison against proper controls, the comparison against other MNPs with different concentrations, the lack of explanation of the results (e.g. not analyzing to which compounds corresponds each FTIR peak or not explaining the reasons why different nanoparticles, synthesized with different extracts, showed different characteristics), and the analysis of biological activity only against some entities (which happens a lot in the antimicrobial studies, where just a few strains are used).

There are already some mechanistic studies, however there is still a long way to completely understand the pharmacological, cellular and molecular biological mechanisms behind the biological activities. The mechanistic knowledge will allow the comparison of biological activities between different chemical entities (for example phyto-AgNPs and standard antibiotics), thus demonstrating their full dermopharmaceutical and cosmetic potential.

One of the major problems is the lack of standard doses for the intended effect. For future application of phyto-MNPs as dermopharmaceutical and cosmetic agents, the dose of each phyto-MNPs necessary for each activity needs to be fully described.

After understanding the underlying mechanisms mediating biological activities of phyto-MNPs, the required dose and fully control the production process, standardization will be possible and phyto-MNPs will finally assume their place as dermopharmaceutical and cosmetic entities.

The next step will be introducing the phyto-MNPs as active ingredient in formulations (such as bandages, lotions, gels, creams, among others) for dermopharmaceutical and cosmetic

applications and run rigorous and extended studies. Besides all the studies mentioned above, toxicological analyses need to be done, in manner of deeply understand the safety profile of phyto-MNPs. *In vitro* studies will be followed by studies with *in vivo* models, that will eventually unlock paths for clinical trials and, finally, phyto-MNPs will enter the dermopharmacy and cosmetic fields.

This review proves that green synthesis of NPs, particularly plant-based synthesis of nanoparticles, is an advantageous alternative to chemical and physical approaches. Phyto-synthesis applied for the synthesis of metal-based nanoparticles are on the arise, since it combines the benefits of plant-mediated nanosynthesis and the biological activities of metals. These advantages, together with the growing concern about dermatological disorders and skin care, lead to great investment by the dermopharmacy and cosmetic fields in the plant-based synthesis of metal-based nanoparticles for topical applications.

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7. Appendices

Table 2 - Characteristics, biological activities and relevant outputs of plant-mediated metal-based nanoparticles

Plant (part)	Extract's nature	Phyto-MNP	Particle size (nm)	Zeta potential (mV)	Dispersity And Aggregation	Polydispersity Index (PDI)	Biological activities	Relevant outputs	Ref
Aerva lanata (L.) Juss. (whole plant)	Aqueous	AgNP	5 - 50 Leaf: 14 Flower: 43.5	- 27.1			**<u>In vitro</u>** **Antimicrobial:** (AgNPs/ciprofloxacin): similar in 3/8 strains *ZOI (AgNPs/free extract): 5.66-fold ↑ *ZOI (AgNPs/$AgNO_3$): 4.6-fold ↑	In vitro stability in physiological media and in phosphate buffer solution of different pH: excellent	[5]
Centaurea pumilio L. (aerial)	Methanolic	AgNP	6 - 8				**<u>In vitro</u>** **Antimicrobial:** *MIC (AgNPS/ free extract): 4.27-fold ↓ MIC (AgNPs/ketoconazole): 1.08-fold ↓ in fungal strain *ZOI (AgNPS/ free extract): 1.54-fold ↑ in bacterial strains *ZOI (AgNPS/ vancomycin): higher in bacterial strains **Antioxidant**: EC_{50} (AgNPs/Trolox): 2.33-fold ↓ EC_{50} (AgNPs/free extract): 1.28-fold ↓ **Other activities:** Anti-elastase IC_{50} (AgNPs/free extract): 1.2-fold ↓ Anti-elastase (AgNPs/free extract): 1.02-fold ↓ Anti-collagenase IC_{50} (AgNPs/free extract): 1.13-fold ↓ Anti-collagenase (AgNPs/free extract): 1.23-fold ↓		[31]
dark *Salvia hispanica* L. (seeds)	Aqueous	AgNP	7 - 12		Aggregation		**<u>In vitro</u>** **Antimicrobial:** (dark/white *Salvia hispanica L.*) AgNPs: higher (green/chemical) AgNPs: higher (AgNPs/ampicillin): similar		[17]

white *Salvia hispanica* L. (seeds)	Aqueous	AgNP	8			(dark/white *Salvia hispanica L.*): wider	**<u>In vitro</u>** **Antimicrobial:** (green/chemical) AgNPs: higher (AgNPs/ampicillin): similar		[17]
Cucurbita maxima Duchesne (petals)	Methanolic	AgNP		- 34 ± 2	Monodispersed	0.3	**<u>In vitro</u>** **Antimicrobial:** ZOI (*C. maxima/M. Oleifera*) AgNPs: higher ZOI (*C. maxima/A. Calamus*) AgNPs: higher ZOI (AgNPs/free extract): higher **Anticancer**: IC_{50} (*C. maxima/M. Oleifera*) AgNPs: 1.01-fold ↓		[35]
Moringa oleifera Lam. (leaves)	Methanolic	AgNP		- 34 ± 1	Monodispersed	0.2	**<u>In vitro</u>** **Antimicrobial:** ZOI (*M. Oleifera/A. Calamus*) AgNPs: higher ZOI (AgNPs/free extract): higher		[35]
Acorus calamus L. (rhizome)	Methanolic	AgNP		- 41 ± 2	Monodispersed	0.3	**<u>In vitro</u>** **Antimicrobial:** ZOI (AgNPs/free extract): higher **Anticancer:** IC_{50} (*A. Calamus/C. maxima*) AgNPs: 1.05-fold ↓ IC_{50} (*A. Calamus/M. Oleifera*) AgNPs: 1.06-fold ↓		[35]
Delonix elata (L.) Gamble (leaf)	Aqueous	AgNP	35 ± 2	- 50.3			**<u>In vivo</u>** **Wound healing:** (AgNPs/control): 1.35 - 5.18-fold ↑ Wound closure: 100% in 17 days		[8]
Iresine herbstii Hook. (leaf)	Aqueous	AgNP	44 - 64		Polydispersed		**<u>In vitro</u>** **Antimicrobial:** *ZOI (AgNPs/kanamycin): 1.43-fold ↑ *ZOI (AgNPs/norfloxacin): 1.07-fold ↑ in 4/5 strains *ZOI (AgNPs/ciprofloxacin): 1.15-fold ↑ in 2/5 strains *MIC (AgNPs/kanamycin): 1,2-fold ↓ in 2/5 strains **Antioxidant:** (AgNPs/free ethanolic extract): higher (AgNPs/ascorbic acid): higher in 2/3 assays		[12]

Prosopis juliflora (Sw.) DC. (leaf)	Aqueous	AgNP	10 - 20		Monodispersed		**<u>In vivo</u>** **Wound healing:** (AgNPs conjugate with Carbopol/ control): 1.54 - 2-fold ↑ (AgNPs conjugate with Carbopol/povidine-iodine): 1.11 - 4.17-fold ↑ Wound closure: 99.9% in 15 days		[6]
Euphorbia milii Des Moul. (leaves)	Aqueous	AgNP	20 - 30		Monodispersed		**<u>In vivo</u>** **Wound healing:** (AgNP ointment/ nitrofurazone ointment): 1.19 - 1.27-fold ↑ (AgNP ointment/control): 1.11 - 1.21-fold ↑ Wound closure: 91.45% in 13 days		[14]
Allium sativum L., *Camellia sinensis* (L.) Kuntze (leaves); *Curcuma longa* L. (powder)	Aqueous	AgNP	6.13 - 8.46				**<u>In vitro</u>** **Antioxidant:** *IC_{50} (*C. longa/A. sativum*) AgNPs:1.04 - 1.37-fold ↓ *IC_{50} (*C. longa /C. sinensis*) AgNPs:1.25 - 2.41-fold ↓ *IC_{50} (*A. sativum/C. sinensis*) AgNPs:1.12 - 1.97-fold ↓ *IC_{50} (*C. longa* AgNPs/rutin): 1.33 - 2.36-fold ↓ *IC_{50} (*C. longa* AgNPs/ascorbic acid): 1.03 - 1.42-fold ↓ *IC_{50} (*A. sativum* AgNPs/rutin): 1.1 - 1.72-fold ↓ *IC_{50} (*A. sativum* AgNPs/ascorbic acid): 1.004 - 1.17-fold ↓ in 4/5 assays *IC_{50} (*C. sinensis* AgNPs/rutin): 1.34-fold ↓ in 1/5 assays *IC_{50} (*C. sinensis* AgNPs/ascorbic acid): 1.04-fold ↓ in 1/5 assays *IC_{50} (green/chemicall) AgNPs:1.7 - 2.2-fold ↓ **Anticancer:** *IC_{50} (green/chemicall) AgNPs: 1.33-fold ↓ IC_{50} (*C. longa /A. sativum*) AgNPs: 1.67-fold ↓ IC_{50} (*C. longa /C. sinensis*) AgNPs: 1.85-fold ↓ IC_{50} (*A. sativum/C. sinensis*) AgNPs: 1.11-fold ↓		[46]

Morinda lucida Benth. (leaf)	Aqueous	AuNP	10 - 140					In vitro stability in the beginning and after 24h of skin exposure: excellent	[27]
Woodfordia fruticosa (L.) Kurz (flowers)	Aqueous	AuNP	13 ± 1.2	- 29.9	Polydispersed	0.029	**<u>In vitro</u>** **Antimicrobial**: (Planktonic cells forming biofilm/preformed biofilm): higher **<u>In vivo</u>** **Wound healing:** (1% ointment gel of AuNPs/povidone iodine 5% ointment): comparable (1% ointment gel of AuNPs/control): 1.4 - 2.33-fold ↑ (1% ointment gel of AuNPs-Carbopol®934/ointment base of Carbopol®934): 1.36 - 1.9-fold ↑ (1% ointment gel of AuNPs/2% ointment gel of the extract-Carbopol®934): 1.15 - 1.19-fold ↑ Wound closure: 93.80% in 12 days		[40]
Coleus forskohlii (Willd.) Briq. (root)	Aqueous	AuNP	5 - 18	- 48.6 ± 0.06			**<u>In vitro</u>** **Antioxidant:** (AuNPs/AgNPs): 1.2-fold ↑ **<u>In vivo</u>** **Wound healing:** (AuNPs/AgNPs)0.2mg/g ointment: 1.04 - 1.29-fold ↑ (0.2mg/g AuNPs ointment /1% soframycin): 1.14 - 1.89-fold ↑ (0.2mg/g AuNPs ointment / control): 1.96 - 7.2-fold ↑ (AuNPs)0.2mg/g ointment wound closure: 100% in 14 days	Contrary to the AgNP, there is no agglomeration.	[34]

	Aqueous	AgNP	5 - 15	- 24.3 ± 0.52			**<u>In vivo</u>** **Wound healing:** (0.2mg/g AgNPs ointment /1% soframycin): 1.09 - 1.47-fold ↑ (0.2mg/g AgNPs ointment /control): 1.88 - 5.6-fold ↑ (AgNPs)0.2mg/g ointment wound closure: 96% in 14 days		[34]
Ziziphora clinopodioides Lam. (leaves)	Aqueous	ZnONP	14.22 - 32.34				**<u>In vitro</u>** **Antimicrobial:** (ZnONPs/antibiotics): higher *ZOI (ZnONPs/standard antibiotic): 1.6-fold ↑ *ZOI (ZnONP/free extract): 2-fold ↑ *ZOI (ZnONP/aqueous Zn nitrate solution): 1.89-fold ↑ *MIC (ZnONP/free extract): 2-fold ↑ *MIC (ZnONP/aqueous Zn nitrate solution): 4-fold ↑ *MIC (ZnONP/free extract): 2-fold ↑ *MBC (ZnONP/aqueous Zn nitrate solution): 5.3-fold ↑ **Antioxidant**: (ZnONPs/BHT): identical **<u>In vivo</u>** **Wound healing:** (ZnNP mixed with Eucerin basal ointment/extract ointment): 1.2-fold ↑ (ZnNP mixed with Eucerin basal ointment/aqueous solution of Zn nitrate ointment): 1.36-fold ↑ (ZnNP mixed with Eucerin basal ointment/tetracycline ointment): 1.15-fold ↑ (ZnNP mixed with Eucerin basal ointment/basal ointment): 2.14-fold ↑ (ZnNP mixed with Eucerin basal ointment/control): 2.14-fold ↑ Wound closure: 75% in 10 days		[28]

Barleria gibsonii Dalzell (leaf)	Aqueous	ZnONP	<80		Slight aggregation		**<u>In vitro</u>** **Antimicrobial:** ZOI (0.03% ZnONP gel formulate with Carbopol/0.2% Zn nitrate solution): similar **<u>In vivo</u>** **Wound healing:** (0.03% ZnONP gel formulate with Carbopol/control): 1.06 - 1.53-fold ↑ (0.03% ZnONP gel formulate with Carbopol/dermazine): very similar 0.03% ZnONP gel formulate with Carbopol wound closure: 94.36% in 20 days		[47]
Punica Granatum L., Juice (fruits)		AuNP	50 - 150	22 ± 2	Monodispersed		**Other activities:** Highest SPF value: 18	Stability over time and under extreme circumstances of pH (4-12) and temperature (25 and 60°C): proved	[15]
Adhatoda vasica Nees (leaf)	Aqueous	ZnONP	10 - 12	- 24.6	Monodispersed	0.175	**<u>In vitro</u>** **Antimicrobial:** *bacterial ZOI (ZnONPs/streptomycin): 1.06-fold ↑ *fungal ZOI (ZnONPs/fluconazole): 1.04-fold ↑ (MBC/MIC): 2-fold ↑ (MFC/MIC): 2-fold ↑ (bacterial/fungal): 2-fold ↓ (nanocream formulation/commercial antimicrobial creams): similar (nanocream formulation/2% commercial antifungal cream): higher **Antioxidant:** (ZnONPs/quercetin): similar		[44]
Artemisia haussknechtii Boiss. (leaf)	Aqueous	ZnONP	50 - 60				**<u>In vitro</u>** **Antioxidant:** *(ZnONPs/free extract): 1.12 - 1.21- fold ↑	Surface: smooth	[2]

Coleus forskohlii (Willd.) Briq. (root)	Aqueous	AgNP	5 - 35		Polydispersed	0.387	**<u>In vitro</u>** **Antimicrobial:** *ZOI (AgNPs/AuNPs) 1.2 to 6.09-fold ↑ ZOI (AgNPs/tetracycline): similar **Antioxidant:** (AuNPs/free extract): 1.25-fold ↑		[11]
		AuNP	10 - 30		Polydispersed	0.445	**<u>In vitro</u>** **Antioxidant:** IC_{50} (AuNPs/AgNPs): 1.67-fold ↓ (AuNPs/AgNPs): 1.04-fold ↑ (AuNPs/free extract): 1.3-fold ↑		[11]
Origanum Vulgare L. (aerial parts)	Aqueous	AuNP	72.9 ± 44.22 - 36.75 ± 16.82	-41 to -47 (basic pH); -22 to -26 (acidic pH)		0.48 - 0.52	**<u>In vitro</u>** **Antimicrobial:** *ZOI (AuNPs/free extract): 2.57-fold ↑ **Antioxidant:** (AuNPs/diluted extract): 1.51-fold ↑		[7]
Olax scandens Roxb. (leaf)	Methanolic	AgNP	20 - 60	-15.2 ± 0.5	Monodispersed		**<u>In vitro</u>** **Antimicrobial:** (green/chemicall) AgNPs: 10-fold ↑ MIC (green/chemicall) AgNPs: 2-fold ↓ ZOI (green/chemicall) AgNPs: 2-fold ↑ (AgNPs/ ampicillin): comparable ZOI (AgNPs/ ampicillin): 1.05-fold ↑ ZOI (AgNPs/free extract): 2.22-fold ↑ **Anticancer:** (green/chemicall) AgNPs: reverse effect (green/chemicall) AgNPs: higher (AgNPs/free extract): higher	In vitro stability in physiological media up to 14 days: excellent	[32]
Mirabilis jalapa L. (leaf)	Aqueous	AgNP					**<u>In vitro</u>** **Antimicrobial:** (AgNPs/free extract): higher *ZOI (AgNPs/free extract): 2.14-fold ↑ in 3/5 strains *ZOI (AgNPs/ZnONPs): 1.6-fold ↑ in 3/5 strains **Antioxidant:** (AgNPs/ZnONPs): 4.57-fold ↑ in 1/3 assays (AgNPs/bimetallic NPs): 1.6 - 1.8-fold ↑ in 2/3 assays (AgNPs/free extract): 1.2-fold ↑ in 1/3 assays		[48]

		ZnONP					**<u>In vitro</u>** **Antimicrobial:** (ZnONPs/free extract): higher *ZOI (ZnONPs/free extract): 1.7-fold ↑ in 4/5 strains *ZOI (ZnONPs/AgNPs): 1.7-fold ↑ in 2/5 strains *ZOI (ZnONPs/bimetallic NPs): 1.9-fold ↑ in 1/5 strains **Antioxidant:** (ZnONPs/bimetallic NPs): 1.6 - 1.8 -fold ↑ in 2/3 assays (ZnO/Ag)NPs: 1.01 - 3.7-fold ↑ in 2/3 assays (ZnONPs/free extract): 1.2-fold ↑ in 1/3 assays		[48]
		Zno/ AgNP (bimetallic NPs)					**<u>In vitro</u>** **Antimicrobial:** (bimetallic/monometallic) NPs: higher (bimetallic NPs/free extract): higher *ZOI (bimetallic/ZnO)NPs: 1.95-fold ↑ in 4/5 strains *ZOI (bimetallic/Ag)NPs: 1.44-fold ↑ *ZOI (bimetallic NPs/free extract): 2.16-fold ↑ **Antioxidant:** (bimetallic NPs/ZnONPs): 2.57-fold ↑ in 1/3 assays (bimetallic NPs/AgNPs): 2-fold ↑ in 1/3 assays		[48]
Azadirachta Indica A.Juss., *Hibiscus rosa-sinensis* L., *Murraya koenigii* (L.) Spreng., *Moringa oleífera* Lam., *Tamarindus indica* L. (leaf)	Aqueous	CuONP	12		Aggregation		**<u>In vitro</u>** **Antioxidant:** *IC_{50} (green/chemical) CuONPs: 1.4 -1.94-fold ↓ (NPs synthesized using *H. rosa-sinensis* and *T. indica*/ascorbic acid): comparable **Anticancer**: (green/chemical) CuONPs: higher	Particle size (green/ chemical) CuONPs: 3.37 - 3.71-fold ↓ Aggregates surface: rough	[42]
Quisqualis indica L. *(flower)*	Aqueous	CuNP	39.3 ± 5.45		Monodispersed		**<u>In vitro</u>** **Anticancer:** IC_{50} (CuNPs/free extract): 4.90-fold ↓ In vivo (CuNPs/free extract) tumor weight, day 29: 3.5-fold ↓		[33]

Panax ginseng C.A.Mey. *(berries)*	Aqueous	AgNP	10 - 20		Polydispersed	0.25	**<u>In vitro</u>** **Antioxidant:** (AgNPs/AuNPs): 1.15-fold ↑ (AgNPs/free extract): 1.16-fold ↑ IC50 (AgNPs/AuNPs): 1.06-fold ↓ IC50 (AgNPs/free extract): 1.03- fold ↓ **Anticancer:** IC_{50} (AgNPs/arbutin): 20.75-fold ↓ IC_{50} (AgNPs/free extract): 19.25-fold ↓ IC_{50} (AgNPs/AuNPs): 16.5-fold ↓	In vitro stability in DW and BSA, after 1 month: excellent	[19]
		AuNP	5 - 10		Polydispersed	0.26	**<u>In vitro</u>** **Antioxidant:** (AuNPs/free extract): 1.01-fold ↑ **Anticancer:** IC_{50} (AuNPs/arbutin): 1.26-fold ↓ IC_{50} (AuNPs/free extract): 1.17-fold ↓	In vitro stability in DW, NaCl, BSA and different pH conditions, upon 1 month: excellent	[19]
Panax ginseng C.A.Mey. *(leaves)*		AuNP					**<u>In vitro</u>** **Antioxidant:** (AuNPs/free extract): higher **Anticancer:** (AuNPs/arbutin): 1.1 - 3.9-fold ↑	In vitro moisture retention activity (AuNPs/ glycerin): very similar	[18]
Acalypha wilkesiana Müll.Arg. *(leaf)*		AgNP	10 - 26		Good dispersion		**<u>In vitro</u>** **Antimicrobial:** *ZOI (AgNPs/free extract): 18-fold ↑		[10]

Panax ginseng C.A.Mey.	Aqueous	AuNP					**<u>In vitro</u>** **Antioxidant:** (AuNPs/ascorbic acid): 1.01 - 1.09- fold ↑ in 2/3 assays (AuNPs/free extract): 1.02 - 1.13-fold ↑ **Anticancer:** (AuNPs/arbutin): 1.5-fold ↓	In vitro (AuNPs/free extract) moisture retention: 1.7-fold ↑ In vitro (AuNPs/glycerin) moisture retention: 1.1-fold ↑	[20]
Clerodendrum infortunatum L. *(leaf)*	Aqueous	Cu-doped ZnONP (MNPs1)	<100				**<u>In vitro</u>** **Antimicrobial:** *bacterial ZOI (MNPs2/ZnONPs): 1.57-fold ↑ *bacterial ZOI (MNPs2/MNPs1): 1.32-fold ↑ *bacterial ZOI (MNPs2/cephradine): 1.3-fold ↑ *bacterial ZOI (MNPs1/ZnONPs): 1.18-fold ↑ *bacterial ZOI (MNPs1/cephradine): 1.23-fold ↑ in 2/4 strains *bacterial MIC (MNPs2/ZnONPs): 6.76-fold ↓ *bacterial MIC (MNPs2/cephradine): 2.31-fold ↓ *bacterial MIC (MNPs2/MNPs1): 5.85-fold ↓ *bacterial MIC M(NPs1/ZnONPs): 1.23-fold ↓ *bacterial MIC (MNPs1/cephradine): 1.78-fold ↓ in 2/4 strains *bacterial (MNPs2/cephradine): higher *fungal ZOI (MNPs2/ZnONPs): 2.06-fold ↑ *fungal ZOI (MNPs2/terbinafine hydrochloride): 1.21-fold ↑ than in 2/3 strains *fungal ZOI (MNPs2/MNPs1): 1-31-fold ↑ in 1/3 strains	Impurities: detected MNPs2/MNPs1 Cu level: higher MNPs1/MNPs2 O level: higher	[21]

Clerodendrum inerme (L.) Gaertn. *(leaf)*		Cu-doped ZnONP (MNPs2)					*fungal ZOI (MNPs1/ZnONPs): 1.91-fold ↑ *fungal ZOI (MNPs1/terbinafine hydrochloride): 1.2-fold ↑ in 1/3 strains *fungal ZOI (MNPs1/MNPs2): 1.13-fold ↑ in 1/3 strains *fungal MIC (MNPs1/ZnONPs): 10.24-fold ↓ *fungal MIC (MNPs1/terbinafine hydrochloride): 1.75-fold ↓ in 1/3 strains *fungal MIC (MNPs1/MNPs2): 1.69-fold ↓ in 1/3 strains *fungal MIC (MNPs2/ZnONPs): 17.89-fold ↓ *fungal MIC (MNPs2/terbinafine hydrochloride): 2.78-fold ↓ in 2/3 strains *fungal MIC (MNPs2/MNPs1): 3.22-fold ↓ in 2/3 strains **Antioxidant:** IC_{50} (MNPs2/ZnONPs): 1.31-fold ↓ (MNPs2/ZnONPs): 1.12-fold ↑ IC_{50} (MNPs1/ZnONPs): 1.24-fold ↓ (MNPs1/ZnONPs): 1.09-fold ↑ (MNPs2/BHT): 1.07-fold ↑ IC_{50} (MNPs2/BHT): 1.15-fold ↓ (MNPs1/BHT): 1.03-fold ↑ IC_{50} (MNPs1/BHT): 1.1-fold ↓ (MNPs2/MNPs1): 1.03-fold ↑ IC_{50} (MNPs2/MNPs1): 1.05-fold ↓		
Camellia Sinensis (L.) Kuntze	Aqueous	AgNP and AgNP capped with PEG		- 35.5 ± 3.32 Capped: 37.03 ± 1.49	Moderate polydispersity	0.28 ± 0.01 Capped: 0.25 ± 0.02	**<u>In vitro</u>** **Antimicrobial:** *MIC (AgNPs/capped-AgNPs): 4.69-fold ↓ in 4/5 strains *MBC (AgNPs/capped-AgNPs): 3.54-fold ↓ in 4/5 strains	Impurities: not detected Capped-AgNPs/ AgNPs level of O: lower Capped-AgNPs/ AgNPs level of C: higher In vitro permeability after 24h of incubation	[43]

Cartoxylum formosum (Jack) Dyer *(1), Phoebe lanceolata* (Nees) Nees *(2), Scurrula parasitica* L. *(3), Ceratostigma minus* Stapf ex Prain *(4), Mucuna birdwoodiana* Tutcher *(5), Myrsine africana* L. *(6), Lindera strychnifolia* (Siebold & Zucc.) Fern.-Vill. *(7) (leaf, stem, root, aerial parts, flower, fruit, seed)*	Aqueous ethanol	AgNP	1: 8.8 ± 0.3 2: 17.7 ± 0.3 3: 26.2 ± 0.7 4: 16.4 ± 0.3 5: 35.4 ± 5.9 6: 11.4 ± 0.1 7: 15.7 ± 1.2	1: -27.81 2: - 29.72 3: - 27.06 4: - 34.56 5: - 33.79 6: - 35.12 7: - 26.78			**<u>In vitro</u>** **Antioxidant:** (AgNP-3/free extract): slightly higher (AgNP-5/free extract): similar (AgNP-6/free extract): similar to slightly higher (AgNP-4/free extract): slightly higher **Wound healing**: (AgNPs-7/other AgNPs): 1.36 - 1.78-fold ↑ (AgNPs-7/control): 1.64-fold ↑ (AgNPs-7) wound closure: 64% in 2 days	Shelf stability, T_{amb}: maintained for 28 days Colloidal stability, T_{amb}: maintained for 28 days	[1]
Abutilon Indicum (L.) Sweet *(leaf)*	Aqueous	ZnONP	<100				**<u>In vitro</u>** **Antimicrobial:** *ZOI (ZnONPs/cephradine): 1.08-fold ↑ in 2/4 strains *MIC (ZnONPs/cephradine): 1.37-fold ↓ in 2/4 strains	Impurities: not detected Nature: homogeneous Surface: equally distributed	[21]

Abbreviations: BHT, Butylated hydroxyl toluene; BSA, Bovine serum albumin; DW, Deionized water; EC_{50}, Half-maximal effective concentration; IC_{50}, Half-maximal inhibitory concentration; MBC, Minimum bactericidal concentration; MFC, Minimum fungicidal concentration; MIC, Minimum inhibitory concentration; NP, Nanoparticle; PEG, polyethylene glycol; SC, Stratum corneum; SPF, Sun protection factor T_{amb}, Ambient temperature; ZOI, Zone of inhibition

↓, Lower; ↑, Higher

By way of comparison, the most efficient concentration of plant-mediated metal-based nanoparticles was selected.

The comparisons against "control" refer to a negative control.

The particle size presented was obtained my microscopy, since the majority of the studies utilized microscopic methods.

* mean values