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Review

Moringa oleifera and their phytonanoparticles: Potential antiproliferative agents against cancer

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ABSTRACT

Cancer is classified as one of the leading causes of global mortality. It has affected millions of people, often with poor prognosis. Having severe side-effects with conventional chemotherapy, alternate drugs and therapies are actively being investigated. There is a need for innovative drug discovery and design as existing cancer therapies are costly and not readily available. Ayurveda and traditional medicine have utilised natural resources such as plants and trees as part of their regime to treat various illness and diseases with positive outcomes. One such tree is *Moringa oleifera* (MO). Almost all parts have shown to be effective against several ailments including cancer which was attributed to the bioactive constituents. Targeted therapies had led to the development of nanoparticles which are extremely effective in various biomedical applications due to their small size. Green synthesis of gold nanoparticles have great potential as naturally occurring plants and trees such as MO can be used in the synthesis process. The resultant gold phytonanoparticles are useful in cancer therapies with improved survival rates and quality of life. The review highlights the importance of MO in natural medicine, synthesis of phytonanoparticles and the fundamental role as a potential antiproliferative agent against cancer.

1. Introduction

Natural traditional medicine, still actively practiced worldwide, has been part of many lives through several generations [1]. Although Western medicine has revolutionised health-care, many pharmaceutically active drugs are being developed based on natural resources (e.g., plants) as they provide many bioactive components [1]. As conventional therapies are relatively expensive and accessibility is a major concern especially in third world countries consisting of huge rural populations, natural plants and trees have provided the necessary resource to supply their demand as either food or medicinal use for treatment of various diseases including cancer [1].

Natural medicinal plants are found throughout the world [2]; natural medicinal products are readily available, easily ingested, relatively non-toxic and cost-effective [2]. Approximately 70% of available and approved drugs have been developed from these natural resources [2]. The use of medicinal plants for formulation is beneficial as their bioactive constituents have an impact on multiple biological signalling pathways [2]. They can act in synergism to produce a desired therapeutic outcome [2]. This can be seen to be effective in comparison to a single dose of a compound [2]. However, investigations into the quality, safety and efficacy of these natural medicines are imperative in the development as alternate therapeutic agents [2].

Novel cancer agents are imperative to combat the existing problem of drug-resistance, side-effects and costs. Natural products have inspired the development of anticancer agents [1]. These include terpene paclitaxel from *Taxus baccata* and Vinca alkaloids from *Catharanthus roseus* [1]. There are many plants that are yet to be explored for their

Abbreviations: ARE, antioxidant response element; CPT1, carnitine palmitoyltransferase I; CPTII, carnitine palmitoyltransferase II; associated 9 (Cas9), clustered regularly interspaced short palindromic repeat (CRISPR); DEN, diethyl nitrosamine; DMBA, 7, 12 dimethylbenz (a) anthracene; GMG-ITC, glucomoringin derived-isothiocyanates; GST, glutathione *S*-transferase; AuNPs, gold nanoparticles; gRNA, guide RNA; HO1, heme oxygenase 1; hnRNPs, heterogeneous nuclear ribonucleoproteins; HDR, homology-directed DNA repair; IAPs, inhibitors of apoptosis proteins; MO, *Moringa oleifera*; NFκB, nuclear factor kappa B; Nrf2, nuclear factor (erythroid-derived 2)-like 2; NQO1, NAD(P)H:quinone oxidoreductase 1; NHEJ, non-homologous end-joining; PAM, Protospacer Adjacent Motif; ROS, reactive oxygen species; Smac/DIABLO, second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low PI; SA, South Africa; AgNPs, silver nanoparticles; SR, Ser/Arg rich; TEM, Transmission Electron Microscopy

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biological activities. *Moringa oleifera* (MO) is a traditional medicinal tree which has shown great potential in complementary and alternate medicine [3]. MO has been used traditionally for the treatment of hyperglycaemia, inflammation, bacterial/viral infections and cancer [4]. MO has a high content of antioxidants and bioactive compounds that play an important role in their efficacy [4].

Nanotechnology has revolutionised modern medicine; it has several characteristics which impact on their mode of action [5]. Nanoparticles are relative small with a large surface area [5]. Green synthesis of nanoparticles utilises plant extracts as reducing agents [5]. The plant used during the synthesis process plays an important role as it contributes/influences the resultant nanoparticle characteristic [5]. The plant extracts, in general, are rich in polyphenols and bioactive compounds which aid in the reduction process [5]. MO is abundantly rich in polyphenols which can be used in the synthesis of phytonanoparticles [5]. This review aims to outline the importance of natural medicines and phytonanoparticles from MO as potential antiproliferative agents against cancer.

2. *Moringa oleifera* – nature's gift

Moringa oleifera (MO), originally found in India [2], is now located across the world including SA [6]. It belongs to the family Moringaceae [6]. It is unique with great potential highlighted by the National Institute of Health [2]. The plant is highly valued as almost all parts are used as a food source, as well as in the traditional treatment of various ailments and to promote good health [2]. These parts include but not limited to the leaves, flowers, seedpods, seeds, roots, bark and gum [2]. It is used traditionally to treat bronchitis, infections and fever amongst several other illnesses [2]. It also displays antioxidant, antibacterial, antifungal, antidiabetic, neuroprotective, cardioprotective and anti-inflammatory properties [2]. MO is known to modulate the immune system [2]. It has also shown to have diuretic and cholesterol lowering activity [7]. In addition, MO is hepatoprotective, increases the rate of wound healing and are antihypertensive [7]. MO improves function of the liver and kidneys and regulates the thyroid hormone [6].

MO belongs to the family *Moringaceae* and is commonly referred to as the Drumstick tree, Miracle tree or Horseradish [7]. There are 13 species however MO is the most cultivated with height ranging between 5–10 m (Fig. 1) [6]. The tree grows rapidly and has drought resistance properties therefore can be grown in tropical, subtropical and arid regions of the world [7].

The safety and efficacy of natural medicinal plants such as MO is imperative for continued traditional and conventional use. The aqueous leaf extract were assessed for safety in rats [9]. Various doses were assessed acutely and chronically. It was determined that intake of MO leaves up to 2000 mg/kg is relatively safe. Asiedu-Gyekye et al., 2014 also assessed acute (5000 mg/kg) and sub-acute (40–1000 mg/kg) toxicity in rats [10]. No adverse effects was seen however there was an elevation in liver enzymes. It was concluded that the leaf extract is safe and consumption should not exceed 70 g/day.

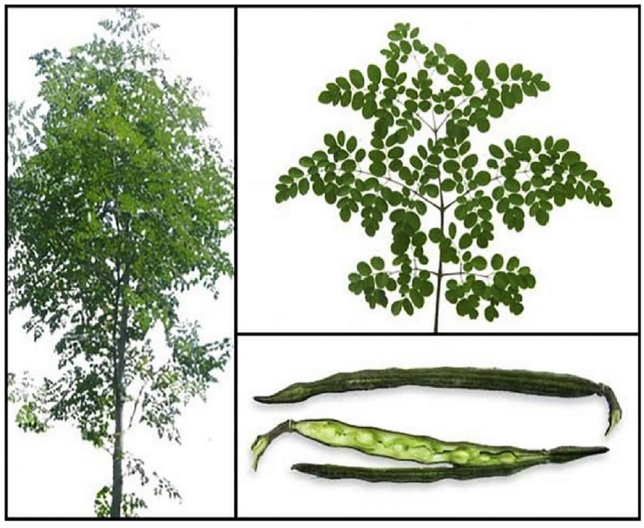

Fig. 1. *Moringa oleifera* tree, leaves, seedpod and seeds [8].

Table 1
Comparison of the nutritional content in MO leaves [2].

Nutritional Content	Nutritional value	Comparison
Vitamin A	4x	Carrots
Vitamin C	7x	Oranges
Potassium	2x	Bananas
Iron	>	Spinach
Protein	>	Egg
Calcium	>	Milk

3. Nutraceutical properties of *Moringa oleifera* – potential use in cancer cachexia

The tree is grown as a food and medicinal source [7]. Due to its nutritional properties, the leaves are used in the herbal formulation of tea [2]. The leaves can also be used raw, cooked, or powdered and still retain its nutritional content. In addition, the leaves are preferred due to the following nutritional properties (Table 1):

The nutritional property of MO varies due to the environment, cultivation methods and genetic background which influences their content [7]. It is a rich source of phosphorous, folic acid, β-carotene and glutamic acid. The leaves contain high levels of nutrients which are required for growth and development which can be beneficial in developing countries where malnutrition is prevalent [6,11]. MO has grown well in both rural and urban settings therefore a vital sustainable source for malnutrition. MO has a good source of phytosterols such as sitosterol, kampesterol and stigmasterol [11] which enhances estrogen production.

High levels of vitamin A, C, and E is present in MO leaves [6]. Vitamin A plays a key role in vision, immunity, cell growth and differentiation and reproduction. Vitamin C and Vitamin E assist in protection against free radicals therefore serve as good source of antioxidants. In addition, MO has a high content of terpenoids, anthraquinones and glycosides [11]. The seedpod is fibrous and therefore aid in digestion. The seedpod, leaves and flowers have 30%, 44% and 31% amino acid content respectively. Oleic, linoleic and linolenic acid are present at 76% in MO seed oils comparable to olive oil.

MO contains a natural source of important bioactive compounds which act synergistically in its therapeutic effect. An inflammatory response in various diseases is a result of an increase expression of nuclear factor kappa B (NFκB), cytokines (pro-inflammatory IL-1β, IL-6 and TNF-α) and nitric oxides [12]. It is also a host defence mechanism. In addition, nuclear factor (erythroid-derived 2)-like 2 (Nrf2) also plays a vital role by upregulating antioxidant and chemopreventive genes. Nrf2 inhibits the inflammatory response. Nrf2 mode of action is a result of exposure to an environmental/chemical stress and increase in reactive oxygen species (ROS) which causes Nrf2 to translocate to the nucleus [12]. It binds to the antioxidant response element (ARE) where it cause the transcription of important genes such as glutathione S-transferase (GST), NAD(P)H:quinone oxidoreductase 1 (NQO1) and heme oxygenase 1 (HO1). Anti-inflammatory drugs have several health risks therefore alternate drugs are investigated. MO seeds have shown to have antioxidant and anti-inflammatory activity due to the nitriles, glycosidic glucosinolates, isothiocyanates, carbamates and thiocarbamates. 4-[(α-L-rhamnosyloxy)-benzyl] glucosinolate (glucomoringin) is found in the seed extract and is converted by myrosinase to form its corresponding 4-[(α-L-rhamnosyloxy)-benzyl] isothiocyanate. Their

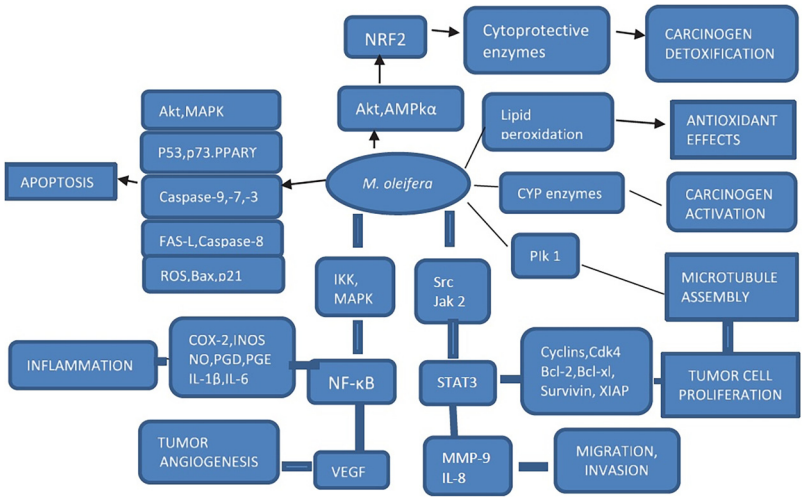

Fig. 2. The anticancer potential and molecular targets of *Moringa oleifera* [25]. (*M. oleifera*: *Moringa oleifera;* Akt: Protein kinase B; AMPkα: 5′ adenosine monophosphate-activated protein kinase α; NRF2: nuclear factor (erythroid-derived 2)-like 2; CYP: Cytochrome P450; PIK1: Phosphatidylinositol 4-kinase; Src: Proto-oncogene tyrosine-protein kinase; Jak 2: Janus kinase 2; STAT3: Signal transducer and activator of transcription 3; MMP-9: Matrix metallopeptidase 9; IL-8: Interleukin 8; IKK: IκB kinase; MAPK: mitogen-activated protein kinases; NFκB: nuclear factor kappa B: VEGF: Vascular endothelial growth factor; COX-2: cyclooxygenase-2; INOS: Inducible NOS; PGD: prostaglandin D; PGE: prostaglandin E; IL-1β: Interleukin 1β; IL-6: Interleukin 6; P53: Tumour suppressor protein p53; p73: Tumour suppressor protein p73; PPARγ: Peroxisome proliferator-activated receptor γ; Caspase-9, -7, -3: cysteine-aspartic proteases -9 (initiator), -7 (executioner), -3 (executioner); FAS-L: FAS ligand; Caspase-8: cysteine-aspartic proteases 8 (initiator); ROS: reactive oxygen species; Bax: Bcl-2-associated X protein; p21: Cyclin-dependent kinase inhibitor).

antioxidant and anti-inflammatory effects is due to the increase in Nrf2, decrease in NFκB and cytokine levels. 4-[(α-L-rhamnosyloxy)-benzyl] isothiocyanate was shown to be more efficient than sulforaphane in the inhibition of NFκB activity [13]. The MO seed isothiocyanate decreased NO, cytokines and significantly increased Nrf2 target genes such as GSTP1, HO1 and NQO1 [12]. The extract shows promise as an anti-inflammatory and antioxidant agent and could be used in various chronic inflammatory diseases.

Wright et al., 2017 showed that MO grown in Jamaica had antioxidant potential [14]. The ethanol water extract of the leaves were assessed for the antioxidant capacity using the DPPH assays. The polar solvent extracts had greater antioxidant activity which has implications in sickle cell anaemia where there is chronic oxidative stress.

Cancer cachexia is a syndrome characterised mainly by weight loss and wastage [15]. In addition, skeletal muscle and adipose tissue are severely depleted. It is mainly seen in cancer patients who have reached the terminal stages of their illness [16]. There are several symptoms of cancer cachexia including muscles wastage, reduced physical and mental performance. Metabolism is also altered thereby affecting lipid, protein and carbohydrate metabolism. The impairment of β-oxidation in the liver leads to the development of cancer cachexia [15]. Inflammatory cytokines viz. TNF-α, IL-6, IL-1β and IFN-γ also play a role. In cachexia, carnitine palmitoyltransferase I (CPT1) and carnitine palmitoyltransferase II (CPTII) activities are decreased. The transport of long-chain fatty acids to mitochondria are compromised and hence liver function is severely impaired. The liver is the most important organ for metabolism and detoxification [17]. Therapeutic intervention for cancer cachexia is very limited; therefore alternative anti-inflammatory and nutritional supplementation therapies are currently being investigated [18]. Severe skeletal muscle degeneration can be alleviated by the intake MO leaves [19]. In an experiment, rats with skeletal muscle degeneration showed that the calcium ATPase activity in their skeletal muscle significantly increased followed a diet of MO leaves [19]. In addition, oxidative stress in these rats was prevented due to MO's high nutritional (Table 1) and antioxidant potential [19].

4. Anticancer potential of *Moringa oleifera*

MO has potential as an anticancer agent against several cancers [6]. MO leaf extract inhibited the cell viability of hepatocellular carcinoma, acute lymphoblastic and myeloid leukaemia [20]. The bioactive compounds responsible for the inhibition were attributed to niazimicin, β-sitosterol-3-O-β-D-glucopyranoside and 4-(α-L-rhamnosyloxy) benzyl isothiocyanate. Berkovich et al., 2013 showed that MO leaves inhibited pancreatic cancer cell growth [21]. It targeted the cell cycle resulting in cell accumulation at sub-G_1 phase. In addition, MO leaves down-regulated the NFκB pathway by decreasing the expression of IκBα, p-IκBα and p65 proteins. It synergistically induced cytotoxicity with cisplatin in pancreatic cancer cells. It is also effective against breast cancer cells [6]. Sadek et al., 2017 showed the cancer preventing effect of MO leaves against liver cancer which was induced by diethyl nitrosamine in rats [22].

Madi et al., 2016 investigated the hot water MO leaf extract which displayed antiproliferative properties in A549 lung cancer cells [2]. The extract increased reactive oxygen species which led to the induction of p53, caspases, and cleavage of PARP-1. This resulted in apoptosis in the cancer cell line. In addition, we showed that the hot water crude aqueous extract also had antiproliferative properties in A549 lung cancer and SNO esophageal cancer cells [3,23]. The extract modulated oxidative stress and induced DNA fragmentation. In addition, the extract upregulated apoptotic markers leading to cancer cell death. MO fruit extract induced apoptosis via the mitochondria in human melanoma A2058 cells [24] by increased ROS production, caspase-9, -3/7 activities and MAPK phosphorylation [24].

Glucosinolates present in MO is effective against cancer [25]. It has the ability to induce apoptosis. Cancer cells rapidly proliferate and therefore anticancer agents are required to target their mode of action. Interestingly, MO has been shown to possess a wide range of activity and can target several proteins and molecules to inhibit cancer cell progression [25]. As shown in Fig. 2, MO has the potential in the development of a novel alternate and complementary therapeutic agent against cancer.

The leaves and bark are effective anticancer agents [26]. It displayed antiproliferative effects in HCT-8 and MDA-MB-231 cancer cell lines. However, the seed extract was not effective. Apoptosis occurred with G2/M phase cell cycle arrest. The anticancer effect was attributed to the bioactive compounds such as D-allose, hexadeconoic acid ethyl ester, eugenol and isopropyl isothiocyanate. This is particularly important as the bioactive compounds identified possessed a sugar moiety, aromatic rings and long chain hydrocarbons. Therefore it can be seen that MO's potential in novel drug development.

5. Chemotherapeutic agents – synergistic effect with *Moringa oleifera*

Approximately 35% of cancer patients in United States of America used herbal medicines [27]. This usage was in conjunction with their chemotherapy and in developing countries it is more than 50% [27]. Complementary, alternative and traditional medicine is often used in third world countries, especially rural areas where accessibility to conventional therapies is poor or non-existent. In addition, affordability of these therapies is a major concern. The interaction between the herb/

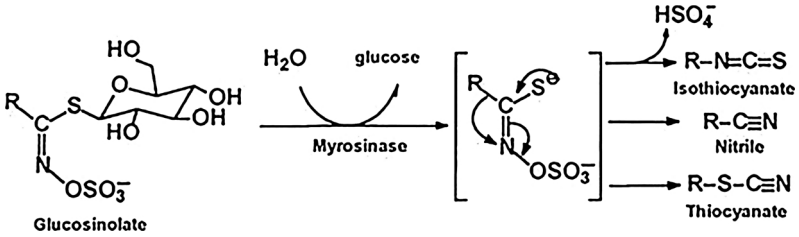

Fig. 3. The conversion of glucosinolate to isothiocyanate, nitrile and thiocyanate via myrosinase [33].

plant (natural products) and a conventional drug is also a concern affecting positive outcomes. Cytochrome P450 enzymes are responsible for drug metabolism, particularly CYP3A4 which influences the therapeutic outcome of the drug [27].

Medicinal plants are either used as a crude or purified extract [28]. Medicinal plants that modulate the immune response have reduced the risk of carcinogenesis. [28]. These plants are rich in phytochemicals and display potent antioxidant and anti-inflammatory activities which offer protection against disease [28]. The high content of flavonoids and vitamins boost the immune system enhancing the immune response via lymphocytes and increased phagocytosis [28].

Sreelatha et al., 2011 indicated that MO leaf induced apoptosis in the KB human tumour cell line [29]. MO leaf extract inhibited cell proliferation which was similar to the anticancer drug cisplatin. Morphological changes and DNA fragmentation in the cancer cells were also observed after treatment with MO and cisplatin following 48 h exposure [29]. MO also acted synergistically with doxorubicin to induce cytotoxicity and enhanced apoptosis in human HeLa cervical cancer cells [30]. Both doxorubicin and MO have the potential to induce apoptosis therefore by their combination it resulted in a stronger synergistic response ensuring the ultimate cancer cell death. It is also suggested the co-administration of MO with a cancer drug can reduce their side-effects resulting in a positive outcome [25].

Complementary and alternate medicine have gained interest in cancer patients as it has shown potential in therapies and have been effective with minimal toxicity [31]. The combination of conventional chemotherapeutic agents with natural products can be beneficial in reducing the negative effects seen in chemotherapy. Many natural products such as MO have been seen as complementary and alternative medicines [3]. Modern western medicines consists of pure compounds with a specific target for therapy [31]. However, natural products consists of a variety of bioactive compounds thereby acting synergistically on a range molecular targets which improves efficacy and survival outcomes [31]. Isolated bioactive compounds have the potential to target various cancer pathways further enhancing a positive outcome.

6. Bioactive compounds from *Moringa oleifera* – novel agents for drug development

Moringa oleifera has shown great beneficial effects in human which can be attributed to their bioactive compounds [6]. MO is a rich source of several phytochemicals [2]. These include phenols, flavonoids, vitamins, minerals, quercetin and kaempferol. In addition, it also contains carotenoids, phenolic acids, alkaloids, glucosinolates and isothiocyanates [6]. The leaves contain higher concentrations of bioactive compounds which contribute to their therapeutic effect.

Flavonoids found in MO leaves have a benzo-γ-pyrone ring in common [6]. Plants produce flavonoids as a protective response against microbial infections. Increased consumption of flavonoids such as quercetin, kaempferol and myrecytin protect against oxidative stress conditions such as cancer and cardiovascular disease. Phenolic acids are formed from hydroxycinnamic and hydroxybenzoic acid [6]. In the leaves, phenolic acids such as Gallic acid are present at high quantities. In addition, caffeic acid and chlorogenic acid are present. Chlorogenic acids play a key role in glucose metabolism. Being an ester of dihydrocinnamic acid it targets and reduces gluconeogenesis and glycogenolysis in the liver by inhibiting glucose-6-phosphate tanslocase. Alkaloids contain nitrogen groups and MO leaves contain the following alkaloids: glucopyranosyl derivative, phenylacetonitrile pyrrolemarumine, 4′-hydroxyphenylethanamide-α-L-rhamnopyranoside and N,α-L-rhamnopyranosyl vincosamide [6]. In addition tannins (water-soluble) and saponins (isoprenoidal-derived aglycone) are present.

Niazimicin found in MO is a bioactive compound that attributes to the anticancer potential [32]. In silico screening showed that niazimicin interacted with ABO system transferase of which the variants are a risk factor for liver and pancreatic cancer. As a result, glycosyltransferases are expressed and are important for various processes. Breast cancer showed increased levels of glycosyltransferases. The product glycan modulates tumour progression. Glycosyltransferases can therefore be a potential target. Niazimicin was shown to interact with glycosyltransferase via hydrophobic interactions and hydrogen bonds. The binding site for glycosyltransferase inhibitors was the same for niazimicin showing potential as an anticancer agent. In addition, the anticancer potential was also confirmed via the Lipinski's rule [32].

Glucosinolates are an important class of bioactive compounds as they are water-soluble, stable and present at high quantities in plants [33]. They are precursors of isothiocyanates. Interesting, upon plant wounding, myrosinase is released causing the glucosinolates to be are converted to isothiocyanates. Myrosinase can also be activated with ascorbic acid. Myrosinase is a β-thioglucosidase which targets the hydroxyl group on the glucose moiety. Glu-426 is required for the catalytic activity. The β-glucosyl moiety is hydrolytically cleaved and the sulfate moiety forms thiohydroxamate-*O*-sulfonate. An intermediate which is not stable, rearranges itself to form the bioactive compound isothiocyanate (Fig. 3). In addition, it can also form nitriles and thiocyanates depending on the reaction conditions (Fig. 3). Furthermore, it can be converted to carbamates and thiocarbamates [12]. MO isothiocyanates is similar to other cruciferous vegetables isothiocyanates. It is comparable to sulforaphane (broccoli) and phenethyl isothiocyanate (watercress) however MO isothiocyanates are much more stable as a result of the sugar moiety. In humans, following consumption of plants, the β-thioglucosidase present in the gut microflora causes the conversation [33].

The leaves, bark and seeds have various bioactive compounds. Cetene is present in the leaves [26]. 5-eicosene, 2-chloropropionic acid and dibutyl phthalate are found in the bark extract. Palmitic acid is found in both leaves and bark. The seeds have 1-octadecene, 1-dodecene, 1-butanamine, 2-decenal, 3-tetradecene and 2-tetradecene. These compounds assist in their therapeutic properties.

7. Green chemistry - phytonanoparticles

Nanotechnology is the combination of chemistry, physics, material science and biology which results in various pharmaceutically important agents [34]. Nanoparticles are relatively small in size (< 100 nm). Green synthesis of nanoparticles has advantages over chemical synthesis. These advantages include environmentally friendly, low cost, simple, reproducible and produces stable nanoparticles [5,35,36]. Gold nanoparticles (AuNPs) are showing potential in medicine due to their size, shape and biological applications [37]. They are highly stable, simple to synthesise and biocompatible. It is favoured

over silver nanoparticles (AgNPs) as AgNPs have toxic effects. AuNPs are used in chemical sensing, catalysis, imaging and drug delivery.

Chemical methods for synthesis of AuNPs have used sodium borohydride and hydrazine as reducing agents [37]. The chemical synthesis involves toxic chemicals, long synthesis procedures, high pressures and temperatures which result in unstable nanoparticles. In addition, upon interaction with a biomolecules, the nanoparticles can aggregate reducing its efficacy. Different size particles are produced which requires further purification. Therefore alternate methods for nanoparticles synthesis are investigated.

Plants extracts such as MO can be used in the green synthesis of nanoparticles. It causes the reduction of metal ions into phytonanoparticles [37]. These synthesised phytonanoparticles are stable with novel structures and biological application. High yields are produced with a control over the shape and size of the phytonanoparticle. Several studies have shown the use of MO in the synthesis of phytonanoparticles. Anand et al., 2015 used MO flowers in the synthesis of gold nanoparticles [38]. The MO flower acted as a reducing agent during the synthesis process. The AuNPs was cytotoxic to A549 lung cancer cells but not cytotoxic to healthy PBMCs. It also showed catalytic activity. The AuNPs reduced nitroaniline and nitrophenol showing potential in the degradation of industrial waste products. The MO flower extract was also used for the synthesis of hydroxyapatite nanoplates [39]. The polyphenolic hydroxyl groups found in the flower extract assisted in the synthesis.

The leaves and roots of MO were used for the synthesis of nanocomposites which showed cytotoxic activity against MCF7, HCT116/Caco2 and $HepG_2$ cell [40]. The leaves was used in a one-pot synthesis of gold phytonanoparticles to assess the antiproliferative properties (Fig. 4) [41]. The AuNPs was successfully synthesised and showed anticancer potential against A549 lung cancer cells with minimal effect on healthy PBMCs. AuNPs activated alternate splicing of *caspase-9* and increased activity and expression of apoptotic molecules in cancer cells resulting in apoptosis. MO leaves were also used in the synthesis of iron-oxide nanoparticles [5]. The green synthesis was environmentally friendly with easy application. It showed potential in the adsorption of fluoride ions in comparison to an adsorbent that is commercially available.

Belliraj et al., 2015 used MO seedpods for the synthesis of AuNPs [42]. The various solvent extracts were screened for their phytochemical composition and revealed the presence of saponins, alkaloids, flavonoids and terpenes. The extracts were further investigated for the potential against bacterial strains [42]. It was effective against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumonia* and *Bacillus subtilis*. Upon synthesis, the AuNPs were crystalline with size of 40–80 nm. The extract and AuNPs were hepatoprotective against carbon tetrachloride, acetaminophen and antitubercular drugs [42].

8. Synthesis and characterization of gold phytonanoparticles

Moringa oleifera leaves were collected and stored at room temperature. The MO leaf extract was prepared by adding 10 g of washed and finely cut leaves in a 300 mL Erlenmeyer flask with the addition of 100 mL of sterile distilled water. The MO leaf extract solution was boiled (10 min), cooled and stored (4 °C); this concoction was used within a week of preparation. A volume of 5 mL of MO leaf extract solution was added to 100 mL of 1 mM aqueous gold chloride solution for the reduction of Au^{+} ions [41]. UV–vis spectra were recorded as a function of the reaction; a colour change to wine red colour within a few hours of mixing the leaf extract with the gold solution supported the formation of AuNPs. Although the mechanism has been adequately elucidated (Fig. 5), the bioactive compounds viz. phenolics, polyols, amines, flavonoids, water-soluble heterocyclic components, reducing sugars and oxido-reductively labile metabolites was able to reduce Au^{+} ions to Au°.

The formation of AuNPs indicated a clear trend on particle size distribution observable as a wider UV–vis spectroscopy. The optical signature of AuNPs were characterised via the distribution of sizes and shapes using Transmission Electron Microscopy (TEM) images followed by the hydrodynamic size of particles (spherical or near spherically

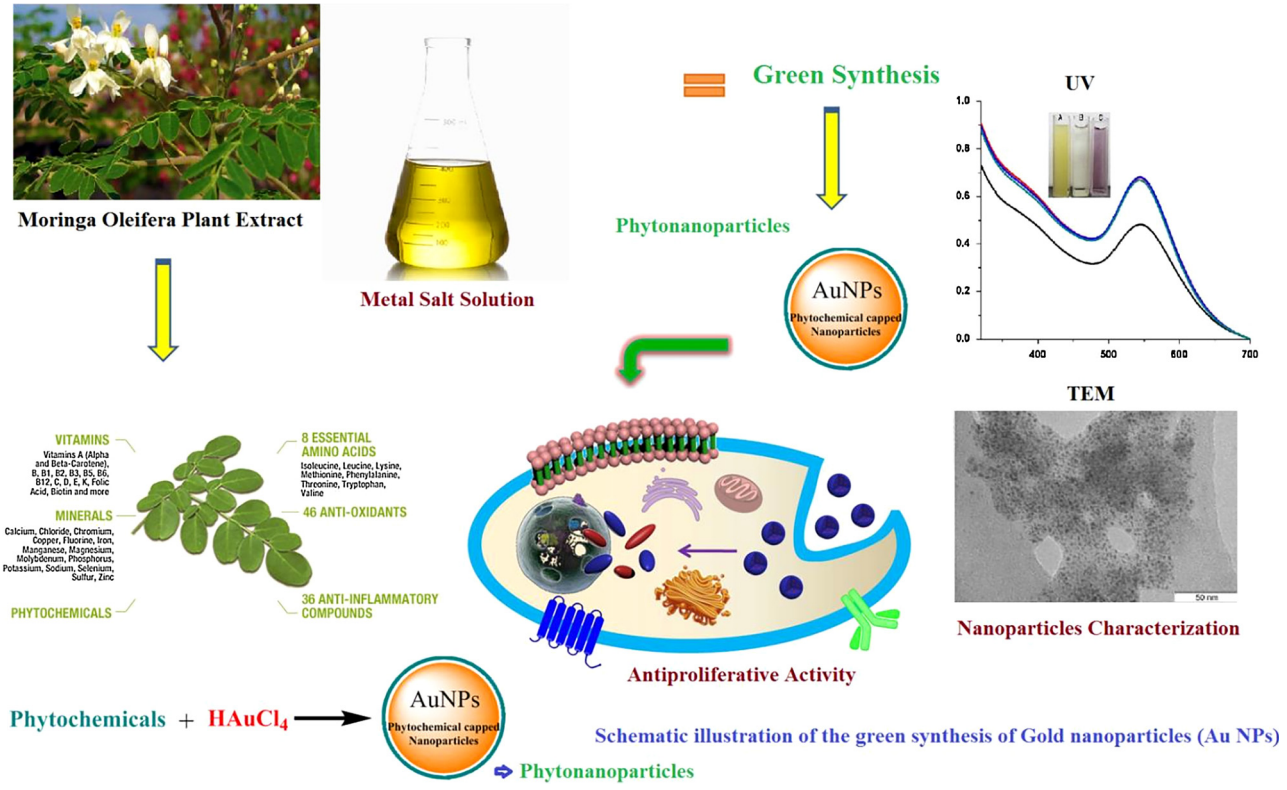

Fig. 4. Schematic illustration of the green synthesis of gold phytonanoparticles.

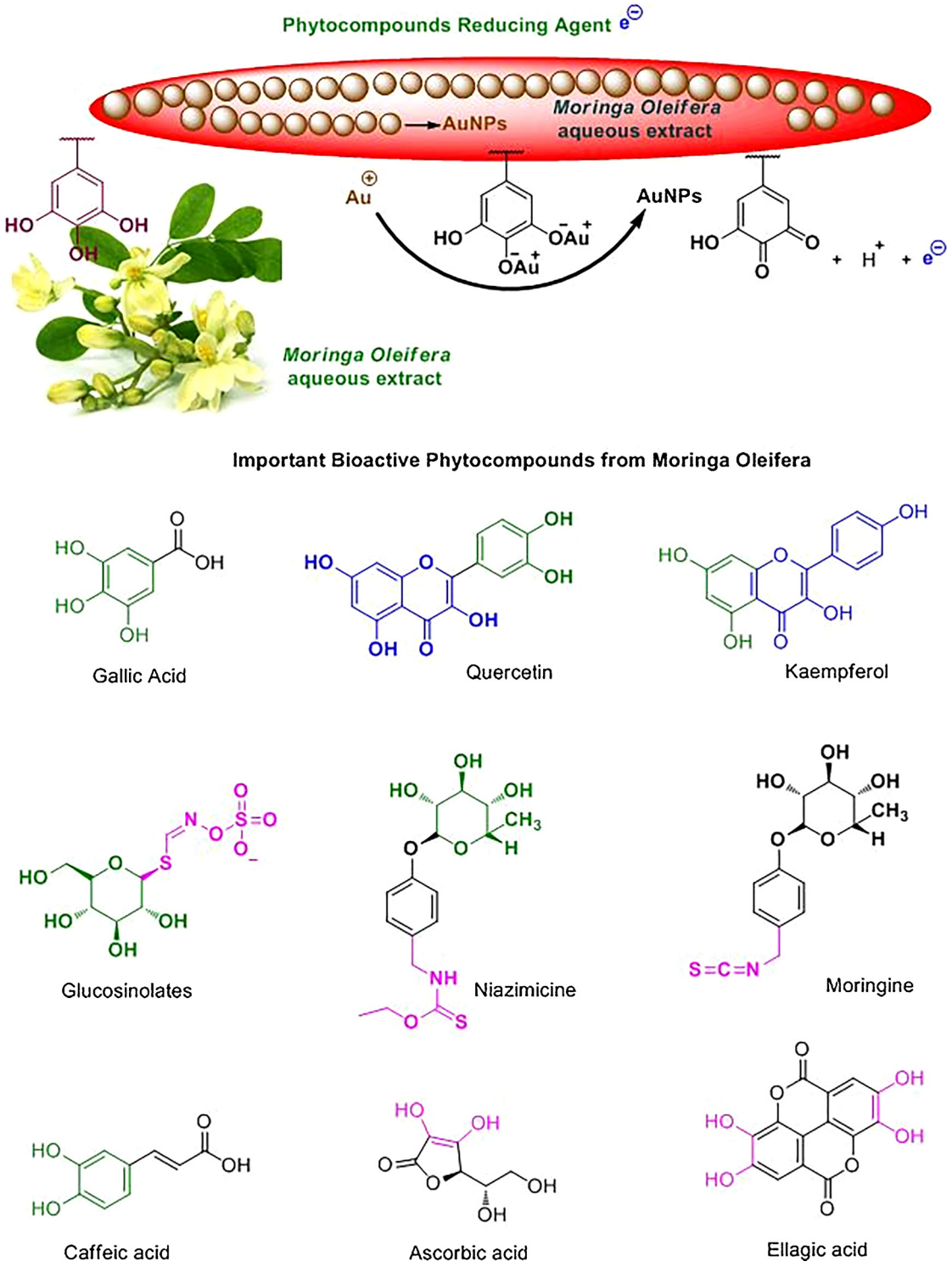

Fig. 5. Mechanism of nanomaterial formation and bioactive compounds from *Moringa oleifera*.

shaped) [38,41,43].

9. Mechanism of nanomaterial formation by *Moringa oleifera* phytochemicals

We have proposed the possible mechanism for poly phenolic interaction with gold metal ions for the phytonanoparticles formation (Fig. 5).

To identify the reducing agents present in the extract which caused the reduction of the metal ions to form nanoparticles, the crude aqueous extract of MO were investigated by GC–MS, NMR and FTIR. GC–MS studies are required to ravel the phytocompounds like hydrolysable Tannins, Saponins, Flavonoids, Glucosinolates and Phenolic acids in the synthesis of AuNPs and a detail mechanism was elucidated in Fig. 4. The compounds which contain the water soluble hydroxy functional group are responsible for the reduction of gold ions and the stabilization of AuNPs. The complex pattern of the ^{1}H NMR spectrum further supports that several compounds were present in the MO extract. The strong signal observed at δ = 1.5–3.81 ppm was due to the single bond and δ = 4.76–4.81 ppm was due to the double bond. Signal

at $\delta = 5.55$ ppm could be due to the aliphatic single bond OH group, whereas the signals appearing between $\delta = 1.26$–1.32 ppm were related to aliphatic −CH2− groups [38,41,43]. FTIR analyses of the MO crude aqueous extract and AuNPs treated spectra showed a stretching frequency shift from 3390 to 3378 cm^{-1} and disappear peak from the flower extract spectrum 2916 and 2848 cm^{-1} suggesting an OH intramolecular hydrogen bond functional group may be responsible for the reduction of Au^{+} to Au°.

10. *In vivo* assessment of the cytotoxicity and anticancer potential of *Moringa oleifera,* bioactive compounds and phytonanoparticles

Hydro-alcoholic MO seedpod extract was used to determine the effect on oxidative stress and chemopreventative potential in mice [44]. Dosages at 125 mg/kg and 250 mg/kg in mice for 7 and 14 days showed that oxidative stress markers levels (lipid peroxidation marker, MDA) was significantly reduced with a concomitant significant increase in antioxidant enzyme activities (catalase, glutathione peroxidase and glutathione reductase) [44]. In addition, liver cytochrome P_{450} activity was significantly increased. The MO extract reduced skin papilloma formation induced by 7, 12 dimethylbenz (a) anthracene (DMBA) and inhibited tumour growth in mice suggesting a possible chemotherapeutic role in carcinogenesis [44]. It showed that MO which is consumed regularly possesses the potential to be a chemopreventative agent. In addition, the safety for MO use is relatively high [44].

Male Wistar rats were used to determine the chemopreventative effect of an ethanolic MO leaf extract against diethyl nitrosamine (DEN)-induced carcinogenesis in rat liver [22]. DEN induced cytotoxicity in the liver with structural changes in hepatocytes and hyperplastic knob formation [22]. Interestingly, the ethanolic MO leaf extract reduced and alleviated the cytotoxicity induced by DEN, in addition to decreasing tumour markers (α-fetoprotein and carcinoembryonic antigen). It also increased apoptosis demonstrating the chemopreventative potential [22]. The hydromethanolic and methanolic extracts of MO leaves and fruits were assessed on mice tumours [45]. The extracts inhibited tumour growth in the mice. However, the leaf extract was able to increase survival time more effectively [45].

Plant flavonoids can be considered as essential to human diets, since humans cannot synthesise flavonoids [46]. An ideal flavonoid rich-plant is MO [46]. The leaves contain quercetin, kaempferol, isorhamnetin and apigenin [46]. MO has a higher concentration of quercetin and kaempferol than spinach [46]. The bioavailability of these compounds depends on the digestion, absorption and metabolism [46]. Investigation into the absorption of quercetin showed that in Wistar rats, the non-glycosidic form is absorbed in the stomach [46]. Currently, there are no dose-response investigations in humans to assess toxicity however, Asare et al., 2012 showed that MO aqueous leaf extract (3000 mg/kg body weight) at high doses was relatively non-toxic and did not influence Sprague-Dawley rat behaviour [47]. Swiss albino mice were used to assess the radio-protective effect of the methanolic MO leaves [48]. Pre-treatment with 150 mg/kg significantly reduced aberrant cell formation; bone marrow chromosomes in the mice were protected against radiation exposure which ensured a higher survival rate [48].

Tamoxifen (an anti-oestrogen receptor-positive inhibitor) is an anticancer drug used extensively for the treatment of breast cancer [49], however patients often experience debilitating side-effects such as endometrial cancer, red blood cell and human serum albumin toxicity [49]. Tamoxifen undergoes first pass metabolism and produces metabolites such as 4-hydroxy-tamoxifen and endoxifen [50]. Cytochrome P450, CYP3A4, CYP3A5 and CYP2D6 isoforms, are responsible for the metabolite formation which contributes to the adverse side-effects and toxicity seen [50]. In addition, genetic variation in the CYP2D6 gene results in reduced metabolism lowering the amount of metabolite available to elicit an anticancer effect [51]. MO has shown to inhibit CYP3A4 and CYP2D6 activity *in vitro* [52]. The administration of MO leaf extract may mitigate the adverse effects following tamoxifen exposure. Rats were exposed to tamoxifen and aqueous MO leaf extract to assess their influence on blood serum levels [49]. Tamoxifen induced mitochondrial dysfunction, ROS production and lipid peroxidation which caused unfolding of serum proteins [49]. There was hydrophobic interaction between tamoxifen and human serum albumin. The administration of MO leaf extract induced an antioxidant response, reducing ROS and the unfolding of the serum proteins thereby protecting against tamoxifen toxicity seen in blood serum [49]. MO was effective in protecting against toxicity without reducing tamoxifen efficacy against breast cancer [49].

MO leaves were fractionated and then assessed for their anticancer potential [53]. The various solvent extracts and fractions showed anticancer potential against Vero and Hep2 cell lines. The dark green F1 fraction (100% Dichloromethane; 5.25% w/w) which contained phenolic compounds and steroids was then investigated further *in vivo* [53]. These fractions showed an increased mean survival time in mice which was comparable to the commercial anticancer drug 5-Fluorouracil [53]. The bioactive compound in MO, Niazimicin, possesses anticancer potential against mouse skin carcinogenesis [45]. MO leaves contain bioactive compounds known as glucosinolates which have apoptosis inducing potential [25]. The hydro ethanolic extract of MO prevented chemical induced carcinogenesis in the livers [25]. Importantly, MO can modulate phase I and II enzymes which are responsible for xenobiotic metabolism and detoxification. Brine shrimp (*Artemia salina Leach*) was used to assess cytotoxicity of MO extract and showed it has potential as an anticancer agent [25].

Plant bioactive compounds such as MO isothiocyanates have shown to activate cytoprotective enzymes and antioxidant pathways [25]. Isothiocyanates activates Nrf2 and phase II enzymes to inhibit cancer formation and cell proliferation [25]. Other bioactive compounds with similar mode of action are quercetin, kaempferol and phenolic compounds [25]. MO glucomoringin derived-isothiocyanates (GMG-ITC) decreased the growth of tumours by regulating the cell cycle in Swiss mice which had A2780 ovarian cancer [13]. It induced caspase-3 activity for the execution of apoptosis.

Phytonanoparticles AgNPs were synthesised via green chemistry using the aqueous MO leaf extract which served both as a reducing and stabilizing agent [54]. The synthesised AgNPs was then assessed in Wistar rats to determine acute toxicity [54]. The results indicated that the phytonanoparticles were relatively safe and could be used further for biomedical applications [54]. Pal et al., 2018 synthesized ZnO nanoparticles using MO leaves and the resultant phytonanoparticles possessed antibacterial properties [55]. In another study, MO leaves were also used in the green synthesis of ZnO nanoparticles [56]. The MO leaf extract served as a chelating agent during the synthesis. Flavonoids, phenolic acids and vitamins found in MO leaves allowed for the conversion of zinc nitrate to ZnO nanoparticles [56]. In addition, bioactive compound N, α-L-rhamnopyranosyl vincosamide was isolated from MO leaves and used for the development of a magnetic hydrogel nanocomposite which displayed a cardioprotective effect in rats [57]. Investigations into AuNPs revealed that the MO leaf extract aided in the reduction of chloroauric acid for the formation of the phytonanoparticles [58]. The AuNPs size ranged from 20 to 60 nm [58]. MO leaf extract stabilized the AuNPs producing a cost-effective, environmentally friendly, and non-toxic phytonanoparticle with various biomedical applications [58]. *In vivo* assessment of novel MO mediated phytonanoparticles and their bioactive compounds are limited and further research into the anticancer potential is imperative which will have an impact on clinical trials.

11. Future drug discovery and design

Novel anticancer agents are being actively investigated as existing therapies are developing resistance. Medical oncologist use chemotherapeutic agents that often have toxic effects on organs despite

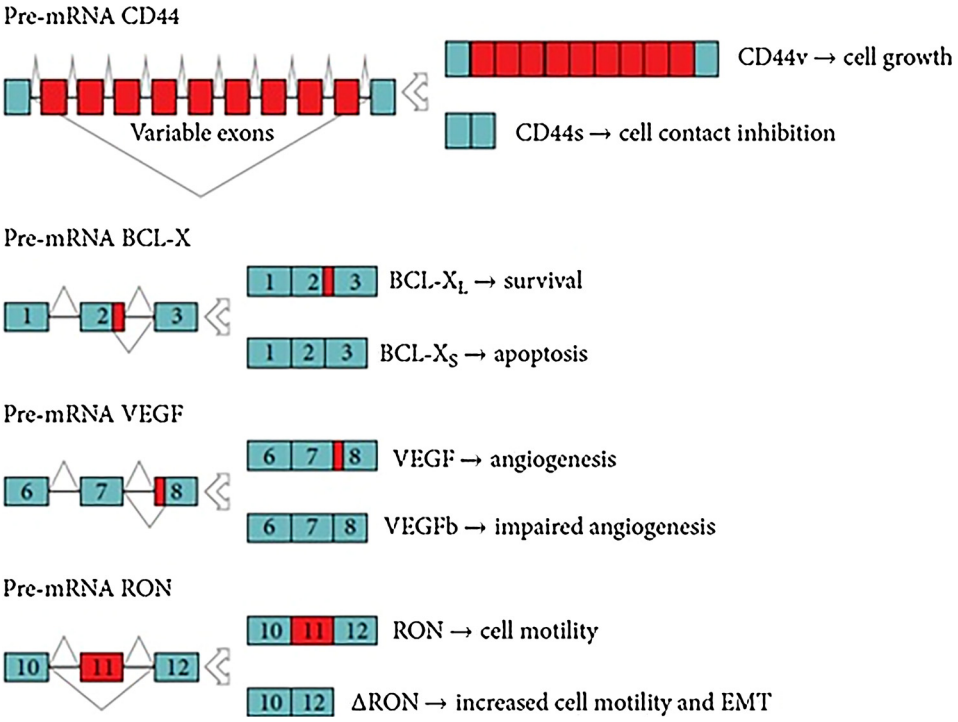

Fig. 6. Alternate splicing resulting in two isoforms with differential functionality [61].

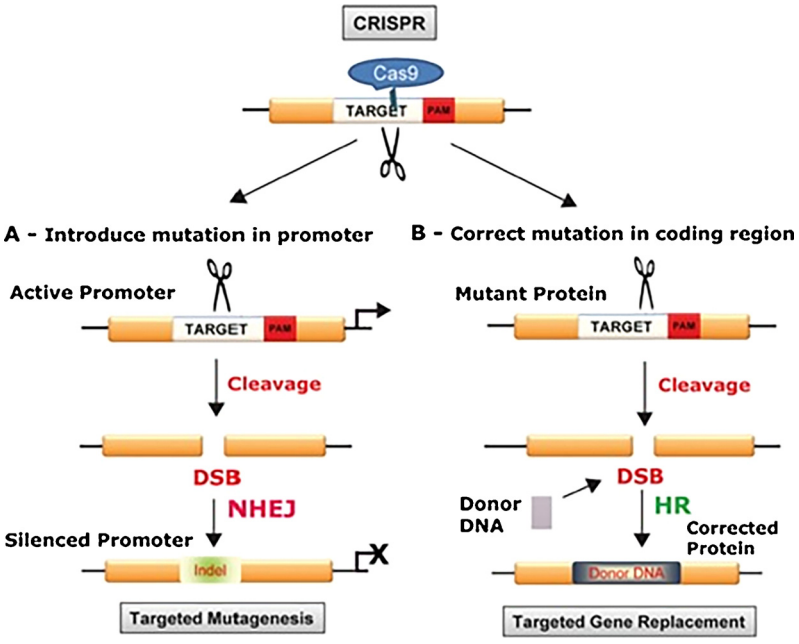

Fig. 7. CRISPR-Cas9 system for genome editing – Introducing/Correcting a mutation [62].

their effectiveness against cancer cells [59]. The efficacy of these drugs is limited resulting in management of adverse drug reactions, resistance and possible treatment-related death. The use of cytotoxic agents in chemotherapy is based on the outcome from clinical trials (Phase 1–3). Pharmacodynamics and pharmacokinetics play a vital role for successful patient outcomes. Therefore, it is imperative to assess during the design and development stage of alternative drugs.

Optimisation of the dosage of a drug is important to determine as high concentrations can result in toxic effects [59]. Ultimately, the drug should have maximum therapeutic effect with minimum toxicity and side-effects. There are several targets for the development of an anticancer agent. Inhibitors of apoptosis proteins (IAPs) are often upregulated in cancers [60]. The development of DEBIO1143 has shown potential due to it being a mimic to second mitochondria-derived activator of caspase/direct inhibitor of apoptosis-binding protein with low PI (Smac/DIABLO). Smac/DIABLO prevents and inhibits IAPs action therefore a potential target as an anticancer agent.

Exon (coding region) and intron (non-coding region) play an key role in the mRNA maturation [61]. The inclusion of exons and removal of introns are referred to as splicing regulated by Ser/Arg rich (SR) proteins and heterogeneous nuclear ribonucleoproteins (hnRNPs). Alternate splicing allows for multiple mRNA splice variants to form from a single gene resulting in differential protein functionality. In cancer there is a dysregulation of alternate splicing causing the formation of

oncogenic splice variants (Fig. 6). This results in cancer cell growth, adaptation to microenvironment and resistance. Therefore alternate splicing can be a possible target in anticancer therapies.

Cancer is characterised by uncontrolled cell proliferation as a result of genetic mutations [62]. Genetic mutations can be a potential target in development of cancer therapies. Clustered regulatory interspaced short palindromic repeat (CRISPR)-associated 9 (Cas9) system is a recent approach to genome editing which has potential against cancers. CRISPR/Cas9 is simple, adaptable and can be used against several targets. Cas9 is an endonuclease with specific DNA targets. The short guide RNA (gRNA) contains a sequence Protospacer Adjacent Motif (PAM) which follows the target sequence (Fig. 7). It recruits the Cas9/gRNA complex to the target. Cas9 is responsible for cutting the DNA strands. The non-homologous end-joining (NHEJ) DNA repair pathway is activated for the repair however it is often leads to insertion or deletions. Homology-directed DNA repair (HDR) can be used to introduce new sequences to the gene. Genomic editing can be used to disrupt oncogenes and repair/correct mutated genes such as those found in tumour suppressor genes [62]. Therefore CRISPR/Cas9 displays potential in targeted cancer therapies.

12. Conclusion

Cancer is a leading cause of global mortality and existing therapies have several side-effects. The cost associated with therapies is rapidly rising which limits access to important life-saving procedures. Alternate therapies are the key in providing more affordable treatment to many. Natural medicine has been a vital source for these alternate therapies. MO has shown potential as an anticancer agent. In addition, these natural medicinal plants can be used for the synthesis of phytonanoparticles with targeted anticancer properties. With advancing medicine these agents can provide a source of easily accessible and affordable therapies in the future.

Competing interest

None.

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