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Molecular insight into the therapeutic potential of phytoconstituents targeting protein conformation and their expression

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ABSTRACT

Background: Native protein conformation is essential for the functional activity of the proteins and enzymes. Defects in conformation or alterations in expression of the proteins have been reported in various diseases.

Purpose: The aim of this study is to review the molecular insight into the therapeutic potential of phytoconstituents targeting protein conformations or expressions.

Methods: Published literatures were searched in PubMed, Scopus, Web of Science; Article published till Dec 2017 were extracted. The literature was assessed from the Central University of Rajasthan, India. Present study evaluate article based on the role of active plant constituents on the conformation and expression of the different proteins.

Results: Plant components play their role either at the molecular level or cellular level and exhibit antibacterial, antiviral, anti-neurodegenerative and other activities. Plant active compounds isolated from different plants may either stabilize or destabilize the conformation of proteins or alter expression level of the protein involved in these diseases, therefore, can play a significant role in preventing diseases caused by the alteration in these proteins.

Conclusion: In the present article, we have reviewed the molecular mechanism of plant active compounds, their target proteins, methods of extraction and identification, and their biological significances. Therefore, a proper understanding of the effect of these herbal molecules on the concerned proteins may help to develop new herbal-based therapeutics for various diseases.

Introduction

Nature has been the source of enormous medicinal agents, and modern drugs have been isolated from traditional medicinal plants. The presence of diverse bioactive molecules in these plants makes them a rich source of medicine (Kothari et al., 2010; Tiwari et al., 2015). It has been reported in the literature that about 80% of the world’s population, mainly the developing countries depend on plants to meet their primary health care (Akinsulire et al., 2007). Plants possess primary and secondary metabolites. Primary metabolites are peptides, carbohydrates or sugar, lipids that are directly used in their synthesized form by plants whereas secondary metabolites are synthesized from primary metabolites (Tiwari et al., 2016). Plant secondary metabolites such as alkaloids, flavonoids, tannins, terpenes etc, have shown to have different properties (Mors et al., 2000; Tiwari et al., 2016).

Current antimicrobial drugs become ineffective due to different acquiring resistance by the bacteria such as antibiotic hydrolyzing enzymes (Tiwari et al., 2012a; Tiwari and Moganty, 2013, 2014; Tiwari et al., 2012b), modified target proteins (Vashist et al., 2011), change in permeability (Vashist et al., 2010), up-regulated metabolisms (Tiwari and Rajeswari, 2013; Tiwari et al., 2012c) and MDR efflux pump systems (Ujjeussi et al., 2013; Verma et al., 2018; Verma and Tiwari, 2018), biofilm formation (Roy et al., 2018; Tiwari et al., 2018b). Hence novel antibacterial compounds are urgently needed with new bacterial targets. Different approaches have been tried (Solanki and Tiwari, 2018; Tiwari et al., 2017a,b, 2018a,c; Tiwari and Tiwari, 2015;) but few of them got the positive result. Some plant active compounds destabilize the structure of various target proteins, resulting in the death of disease-causing bacteria (Cowan, 1999; Ernawati et al., 2013; Tyagi et al., 2015) while some active phytochemical compounds alter the affinity of...
bacterial toxin or cell to the receptor of the host cell (Rafsanjany et al., 2015). Traditional herbal medicines can also act as a potential antiviral and antifungal agents that provide molecules with drug-like properties and incredible structural diversity (Rajibhandari et al., 2009). Active phytoconstituents can work either at the cellular or molecular level. Plant-derived molecules should target biochemical features of invading pathogens, which are not possessed by the normal host cell (Samy and Gopalakrishnakone, 2010; Zhang et al., 2012).

Various diseases are associated with the altered conformational change in the native proteins or required correct conformation of receptor and ligand essential for pathogenicity. The modified structure of the native protein might also result in different enzymatic activity in natural enzymes (Tiwari, 2016). The plant consists of multiple compounds like glycocides, alkaloids, terpenoids, etc which are known to exert therapeutic effects (Kothari et al., 2010). Therefore, it is imperative to review the impact of herbal-active compounds on the conformation stability and expression of the proteins. In the present review, we have presented various herbal active compounds that stabilize and destabilize proteins associated with different diseases. We have also reviewed molecules that alter the expression of the various proteins related to pathogenesis. Hence, a detailed understanding of this topic might result in the development of novel drugs or new herbal active compounds. Here, we have highlighted various phytoconstituents that can alter the stability and expression of proteins involved in the prokaryotic systems, proteins that alter during neurodegenerative diseases, proteins that have an essential role and involved in cell signalling. Understanding these alterations in the stability and expression of such proteins might lead to therapeutics to control conformation and expression associated with pathological or diseased conditions.

**Effect of phytoconstituents on the stability, and expression of prokaryotic proteins**

Several proteins have been identified in prokaryotic organisms that act as a target for antibiotics. Conformation stability or expression of these proteins are also influenced by the plant active compounds, hence, can be used as an alternative mechanism to cure these diseases. Some of the plant-derived compounds, and their effects are described in Table 1. Here, we have discussed the role of various plant active compounds with insight into their mechanism of action.

_Curcuma domestica_ contains active compound curcumin that is widely known as an anti-inflammatory (Bi et al., 2016; Zhao et al., 2016), anticancer (Gu et al., 2016; Sufi et al., 2016; Tong et al., 2016) and recently antimicrobial agent (Rahayu et al., 2013; Tyagi et al., 2015). Curcumin correlates with its ability to bind to the vitamin D receptor (VDR) as a potential ligand. Binding of curcumin promotes expression of cathelicidin, an antimicrobial peptide and kills the bacteria. Cathelicidin is a small peptide with some structural similarities with other antimicrobial proteins, such as defensin. Curcumin also increases mRNA expression of _cAMP_, which in turn increases cathelicidin level in tissues thereby curing infections caused by _Salmonella typhi_. Moreover, curcumin also exhibits antibacterial activity against a number of pathogenic bacteria such as _Staphylococcus aureus_, _Staphylococcus epidermidis_, and _Enterococcus_ by damaging their bacterial membrane (Tyagi et al., 2015). FtsZ is a prokaryotic homolog of eukaryotic cytoskeletal protein tubulin and polymerizes to form a Z-ring at the mid-cell, which orchestrates cell division in bacteria. This compound strongly inhibits the formation of cytokinet Z-ring in _B. subtilis_ without detectably affecting the segregation and organization of the nucleoids. Assembly dynamics of FtsZ protofilaments play a significant role in the formation and functioning of Z-ring. Curcumin inhibits the assembly of FtsZ protofilaments, and also increases GTPase activity of FtsZ, thereby affecting bacterial proliferation and finally death (Rai et al., 2008).

Similarly, _Zingiber officinale_ contains Zingerone, which is the critical component of the pungency of ginger. Zingerone is active against enterotoxigenic _Escherichia coli_ (ETEC), which have heat-labile enterotoxin (LT) (Arlt et al., 2013) that induces diarrhea. The heat-labile enterotoxin is the major virulence factor of ETEC. Gm1 cell surface receptor is the docking site for choler toxin and enterotoxin of _E. coli_ (Arlt et al., 2013). Gm1 is the “prototype” ganglioside that has one sialic acid residue. LT (heat-labile enterotoxin) consists of one ‘A’ subunit for catalytic activity and five ‘B’ subunits for binding to the receptor. Binding of B-subunit (LT-B) of the toxin to Gm1 of the intestinal cell surface induces a conformational change in the toxin. This is followed by translocation of A-subunit (LT-A) into cells. Inside intestinal cells, LT-A catalyzes ADP-ribosylation of stimulatory GTP-binding protein that results in increased intracellular levels of cyclic-AMP. The elevated levels of c-AMP in the cells result in massive loss of fluid and ions from cells leading to diarrhea. Zingerone binds at the binding sites of Gm1 receptor and disrupting the interaction, hence A-subunit cannot be transported, finally curing diarrhea (Chen et al., 2007).

Flavonoids are the major components of the extracts from acaceous plants _Acer truncatum bunge_. FabG (beta-oxoacyl-ACP reductase) enzyme participates in the polysaturated fatty acid biosynthesis in bacteria. Polyphenol binding interferes with the binding of the cofactor to the enzyme and thereby destabilization of FabG reductase, which refers to dysfunctioning of fatty acid biosynthesis of Gram-positive and Gram-negative bacteria (Zhang et al., 2008).

Another herbal active compound, Capsaicin (N-anilyl-8-methyl-nonanenamide) of _Capsicum annuum_, acts as an antimicrobial agent against bacterial pathogens such as _Bacillus spp._, _Helicobacter pylori_ etc. It inhibits the production of choler toxin by H-NS mediated inhibition of transcription of virulence genes i.e _ctx_ and _tcpA_ genes. Histone-like nucleoid structuring protein (H-NS), the product of _hns_ gene, is a global prokaryotic gene regulator that represses the transcription of several virulence genes. Capsaicin stabilizes the H-NS which in turn suppress the virulence gene responsible for the synthesis of choler toxin in _Vibrio cholerae_ strains (Yamazaki et al., 2011).

Similarly, apigenin (4, 5, 7-trihydroxyflavone) of _Morinda citrifolia_ L., belonging to flavone class of natural products, that is aglycone of several naturally occurring glycosides. The mechanism of inhibition is interfered by the structure of the flavonoid molecules that can form bonds with the enzyme GTF (glycosyltransferases). GTFs are enzymes that produce glycosidic linkages in various bio-molecules, including cell wall components, natural products, saccharides, proteins, and even nucleic acids. They can also catalyze the transfer of saccharide or monosaccharide moieties from activated nucleotide sugar to nucleophilic glycosyl acceptor molecule with oxygen, carbon, nitrogen, or sulfur nucleophile. Flavones and flavonols have unsaturated double bonds in the chain C-2 and C-3 to form a chain that can be occupied by nucleophilic amino acids of the GTF, especially side chain of aspartic acid. It inhibits GTF formation, which will reduce the adhesion and aggregation or accumulation of _Streptococcus mutans_ colonies and prevents human oral cavity (Ermawati et al., 2013).

 Likewise, epigallocatechingallate (EGCG), the most abundant catechins in tea belong to the class of polyphenols, which have high antibacterial activity. Various cell surface proteins such as oligopeptide ABC transporter binding lipoprotein, phosphate ABC transporter substrate-binding protein, the glucose phosphotransferase system transporter protein, and penicillin-binding protein-5 are responsible for glucose uptake and cell shape in Gram-positive bacteria, _Bacillus subtilis_. TEM (transmission electron microscopy) analysis revealed aggregating forms of these cell surface proteins and EGCG throughout the cell envelope provided evidence that surface proteins act as the target for EGCG. Alteration in the cell shape and glucose uptake after treating the bacteria with EGCG suggested that it interferes with the primary functions of these proteins, leading to growth inhibition of _B. subtilis_ (Nakayama et al., 2015).

_Artimisia annua_ is known to possess an active compound named artemether. This compound has the highest binding affinity towards ENR (Enoyl acyl carrier reductase) involved in fatty acid synthase 2 pathway.
### Table 1.
Effect of the herbal active compounds on the stability and expression of prokaryotic proteins.

| S.No | Plant | Active molecule | Method of extraction | Target protein | Effect on protein | Function | Reference |
|------|-------|----------------|----------------------|----------------|-------------------|----------|-----------|
| 1.   | Curcuma domestica | Curcumin | N/A (Purchased from Sigma-Aldrich) | Cathelicidin | Upregulation | Binding of curcumin to vitamin D receptor activates cathelicidin which kills bacteria | (Rahayu et al., 2013) |
| 2.   | Zingiber officinale | Zingerone | N/A (Purchased from Sigma-Aldrich) | GM1 receptor | Destabilize | Disturb the interaction of GM1 and Heat-labile enterotoxin prevent from diarrhea | (Chen et al., 2007) |
| 3.   | Acer truncatum Bunge | Polyphenols | Ethyl acetate extract | FabG (beta-oxoacyl-ACP reductase) | Destabilize | Interfere with the binding of the cofactor to the enzyme which inhibits the bacterial type II fatty acid synthesis system | (Zhang et al., 2008) |
| 4.   | Capsicum annuum | Capsaicin | n-hexane and 90% methanol extracts | Histone-like nucleoid structuring protein (H-NS) | Stabilize | Increased expression of H-NS & repress the transcription of virulence genes toxT, ctx and tcpA for the cholera toxin production | (Yamasaki et al., 2011) |
| 5.   | Morinda citrifolia | Flavones (apigenin) | 70% ethanol | Glucosyltransferase enzymes | Destabilize | Interact with the enzyme GTF which will reduce the adhesion and aggregation or formation of Streptococcus mutans colonies | (Ernawati et al., 2013) |
| 6.   | Camellia sinensis | Catechins (Epigallocatechin gallate) | NA | Cell surface proteins: oligopeptide ABC transporter binding lipoprotein, glucose phosphotransferase system transporter protein, etc | Destabilize | Reduces deposition of protein and IEGG on cell envelope, showing inhibition of the major functions of these proteins, leading to growth inhibition of B. subtilis. Perturbation of the GTPase activity of FtsZ, and inhibits bacterial cell proliferation | (Nakayama et al., 2015) |
| 7.   | Curcuma longa | Curcumin purchased from Sigma | NA | FtsZ | Destabilize | Perturbation of the GTPase activity of FtsZ, and inhibits bacterial cell proliferation | (Rai et al., 2018) |
| 8.   | Berberis aquifolium | Berberine | NA | NorM protein | Destabilize | Disturb proton motive force thus showing a potent role in drugs therapy | (Long et al., 2008) |
| 9.   | Allium cepa | Quercetin | NA | DNA gyrase | Destabilize | Inhibit ATPase activity of DNA Gyr B | (Piper et al., 2003) |
| 10.  | Larix sibirica | Taxifolin | NA | Fumarate reductase flavoprotein (FrdA) | Destabilize | Decrease anaerobic respiration metabolism and cellular energy synthesis | (Xiao et al., 2014) |
| 11.  | Artemisia annua | Arteether | NA | Enoyl acyl carrier reductase (ENR) | Destabilize | Inhibit the ENR dependent pathway of Mtb | (Dhivya et al., 2011) |
of Mycobacterium tuberculosis. ENR is a part of type II fatty acid synthesis system and plays an essential role in the metabolism. It is an attractive target for antibacterial drug discovery as its sequence is conserved across many bacterial species. In-vivo and in-vitro studies revealed that it could be a useful drug candidate against tuberculosis (Dhiya et al., 2011).

Earlier studies suggest that berberine is a guanetidion ammonium salt from the protoberberbine group of isoquinoline alkaloids that play a significant role in the destabilization of protein consequently killing the bacteria. N. gonorrhoeae contains the NorM efflux pump, which is a member of multidrug and toxic compound extrusion (MATE) family of efflux transporters. MATE family efflux pump, use proton motive force (PMF) energy for export, and loss of the PMF inactivates pump activity and resulting in the enhanced accumulation of substrates. This accumulation increases susceptibility to the agents commonly recognized and effluxed by the transporter. The N. gonorrhoeae NorM transporter is homologous to efflux pump of Vibrio para-haemolyticus and YdhE of E. coli, when expressed in the E. coli; it enhances the efflux of various antibiotics. When berberine expressed to recombinant E. coli with NorM or YdhE as the transgene, both transporters enhanced antibiotics accumulation in the cell through a mechanism requiring the proton motive force. Hence, it can be said that these transporters bind to berberine with dissociation constants in the micro-molar range and destabilize the efflux pumps, therefore, showing a strong role in drug therapy (Long et al., 2008).

Flavonoids exhibit diverse antibacterial activity. Quercetin, an abundant natural flavonoid of Allium cepa inhibits bacterial gyrase that relieves strain during unwinding of double-stranded DNA. Quercetin inhibits gyrase via either its interaction with ATP binding site of DNA gyrase enzyme or target DNA (Plaper et al., 2003). It binds to the ATP binding pocket of 24 kDa fragment of gyrase B of E. coli (Kd of 15 μM) and inhibits ATPase activity of Gyr-B by competitively inhibiting ATP binding. Novobiocin, an amino-coumarin, also inhibits energy transduction of DNA gyrase by binding at ATPase active site, located on the GyrB subunit. Another flavonoid, taxifolin is reported to exhibit strong binding affinity for bacterial protein. The fumarate reductase flavoprotein (FrdA), is a membrane-bound flavoprotein, which catalyzes the interconversion of fumarate and succinate, that is important for anaerobic growth using non-fermentable substrates. Molecular docking studies revealed that flavonoids derivatives (such as galloyl or glycosides substitution at position 3 of heterocyclic pyrane ring) enhance its binding affinity to FrdA, Pyrd (dihydroooretate dehydrogenase) and FabI (NADH-dependent enoyl-ACP reductase) (Xiao et al., 2014).

Different herbal active compounds with their respective plants and their mechanism of action have been listed in Table 1 and Fig. 1. This table showed that numbers of the herbal active molecules shown to have antibacterial activity against various pathogen microorganism, table showed that numbers of the herbal active molecules shown to have antibacterial activity against various pathogen microorganism, and they might be further investigated to have antibacterial activity against various pathogen microorganism, (FrdA), is a membrane-bound substitution at position 3 of heterocyclic pyrane ring) enhance its flavoprotein and transports thyroxine (T4) and retinol. Stabilization of TTR tetramer by small molecules can inhibit amyloid fibril formation of TTR. CAPE inhibits the amyloid fibril formation of TTR and stabilizes the TTR tetramer thus prevent amyloidosis (Yokoyama et al., 2014).

Another molecule, vanillyl alcohol is derived from vanillin, which is a phenolic aldehyde, found to be useful in some neurodegenerative diseases such as Parkinson’s disease. Vanillyl alcohol (VA), a bioactive compound of Gastrodia elata, induced PARP (Poly ADP-ribose polymerase) cleavage in 1-methyl-4-phenylpyridinium-treated MN9D dopaminergic cells (classic model for Parkinson’s disease). PARP is a family of proteins involved in many cellular processes such as DNA repair and programmed cell death. It was found that protective effect of VA is associated with decreased reactive oxygen species levels is downstream apoptotic signaling pathways (i.e decrease in the Bax/Bcl-2 ratio) that prevent proteolysis of PARP by caspases, thereby protect from neurodegenerative diseases (Kim et al., 2011).

Baicalin is one of the essential flavonoids that possess anti-inflammatory and anti-oxidant properties. It stabilizes an unfolded conformation and decreases fibril formation of alpha synuclein that localizes within presynaptic terminals in the CNS. Alpha-synuclein is an intrinsically disordered protein and active in an unfolded conformation. Baicalin causes an increase in the annular oligomers and prevents amyloid formation (Jiang et al., 2010). Studies also revealed the cell cycle arrest and apoptotic activity of wogonin, baicalin and baicalein (Gao et al., 2011). Hence, baicalein and anthraquinone act as a drug for neurological diseases by inhibiting the fibril formation of α-synuclein and insulin respectively. These examples suggest that plant active molecule can be seen as a future hope to screen the molecules that control the neurodegenerative diseases.

Effect of phytoconstituents on the stability, and expression of neurodegenerative disorders associated proteins

A number of drugs are available for various neurodegenerative disorders such as Parkinson’s disease, Alzheimer’s disease, etc. Their high cost and limited effectiveness are one of the major concerns for developing countries (Murphy et al., 2012). Therefore, it is very significant to find alternate herbal-based drugs for these diseases. The neurological disorders have been developed due to the alteration/defect in the conformation or altered expression of various proteins such as amyloid, presenilin, etc. The altered defective protein causes diseases. Hence the molecules that change the conformation of defective proteins (or reverting it to native conformation) might be used as herbal drugs (Table 2). The molecules with their effect on conformation of such proteins are summarized in Fig. 2.

Quinones are the oxidation product of polyphenols, and include benzoquinones, naphthoquinones, anthraquinones, and phenan-thraquinones. Anthraquinone strongly inhibits the aggregation of beta-amyloid protein. Molecular simulations studies have suggested that quinone may destabilize intermolecular cross-beta strand of ‘oligomers’ and ‘mature fibrils’ by breaking the hydrogen bonds, and polar interactions respectively. Quinone may also tightly interact with carbynoyl oxygen and amide hydrogens of peptide backbone via π-δ interactions and hydrogen bonds. Attenuated oligomerization or fewer amyloid fibrils were observed after quinone treatments, which might also suggest its inhibitory role in the oligomerization of amyloidogenic proteins (Gong et al., 2014) such as prion, lysozymes, and insulin. This anti-amyloid property of quinones might also be investigated to prevent beta-amyloid formation in Alzheimer’s disease.

Presenilin, a member of the γ-secretase complex, is also involved in the familial Alzheimer’s disease caused by activating the beta-amyloid formation. Curcumin down-regulate presenilin protein by activating its proteasome-mediated degradation, hence, might play a significant role in curing this neurodegenerative disease (Yoshida et al., 2011). Similarly, caffeic acid phenethyl ester (CAPE), a natural phenolic chemical compound, is the ester of caffeic acid and phenethyl alcohol, and found in Eucalyptus globus. Transthyretin (TTR) is a homotetrameric protein found in the human blood plasma, where it binds to retinol-binding protein and transports thyroxine (T4) and retinol. Stabilization of TTR tetramer by small molecules can inhibit amyloid fibril formation of TTR. CAPE inhibits the amyloid fibril formation of TTR and stabilizes the TTR tetramer thus prevent amyloidosis (Yokoyama et al., 2014).

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Bacaline is one of the essential flavonoids that possess anti-inflammatory and anti-oxidant properties. It stabilizes an unfolded conformation and decreases fibril formation of alpha synuclein that localizes within presynaptic terminals in the CNS. Alpha-synuclein is an intrinsically disordered protein and active in an unfolded conformation. Baicalin causes an increase in the annular oligomers and prevents amyloid formation (Jiang et al., 2010). Studies also revealed the cell cycle arrest and apoptotic activity of wogonin, baicalin and baicalein (Gao et al., 2011). Hence, baicalein and anthraquinone act as a drug for neurological diseases by inhibiting the fibril formation of α-synuclein and insulin respectively. These examples suggest that plant active molecule can be seen as a future hope to screen the molecules that control the neurodegenerative diseases.

Effect of phytoconstituents on the stability, and expression of constitutive essential proteins

In addition to the protein involved in the pathogenesis, there are various constitutive proteins which are involved in the survival of organisms. Alteration in the conformation of the proteins leads to diseases listed below. Some herbal active compounds are identified which can be used to cure these diseases by regaining the normal conformation of these constitutive proteins (Table 3). The herbal active compounds and their effect on the conformation of constitutive essential proteins are
summarized in Fig. 3.

Triptolide, a diterpenetriepoxide, has been widely used as a natural medicine in the treatment of autoimmune as well as inflammatory diseases, including rheumatoid arthritis. Heat shock proteins (HSP), are highly conserved proteins and play a significant role in protecting cells from adverse environmental, chemical, and physical stresses. HSP-70 and HSP-90 are two identified HSPs that can be used as targets for the treatment of cancer. HSP-70 is the significant stress-inducible HSP, which renders cells resistant to several chemotherapeutic drugs. Increased level of HSP-70 in the cancerous cell has shown to confer resistance to apoptosis (Phillips et al., 2007). Triptolide possibly interacts with the regulatory elements caspase-3 which are responsible for elevated expression of HSP-70 in cancer cells that may be different from that involved in the overexpression of HSP-70 during the heat shock response. It decreases the mRNA level of HSP-70 that increases the cyt-C release and in turn induces apoptosis. Similarly, HSP-90 is a highly abundant molecular chaperone in the stress response; include trans-membrane tyrosine kinases metastable signaling proteins, mutated signaling proteins, chimeric signaling proteins, cell cycle regulators and steroid receptors (Phillips et al., 2007). Similarly, epigallocatechin-3-gallate (−EGCG), the most abundant polyphenoliccatechin, shows chemoprevention and anticancer activities. This molecule binds to the C-terminus of HSP-70 at the region of amino acids 538–728 on Hsp-90. Binding of EGCG to HSP-90 impairs the association of HSP-90 with its co-chaperones (HSP-70 and p-23), thereby inducing degradation of HSP-90 client proteins, resulting in anti-proliferative effect in pancreatic cancer cells (Li et al., 2009).

Likewise, tannic acid, epigallocatechingallate (EGCG), epicatechingallate (ECG) and gallic acid belong to tannins galloyl groups. The former three compounds bind to collagen (the fibrous protein found in skin) via extensive hydrogen bonding enhanced by some hydrophobic interactions and prevent the free access by collagenase to active sites on the collagen chains and protect the collagen degradation from collagenase (McRae and Kennedy, 2011).

Quillaja (saponins) is a complex mixture comprising of triterpenoidaglycon and a complex array of oligosaccharides and acylated residues. Quillaja tightly binds to beta-casein, which is a phospho-protein commonly found in milk, which supplies carbohydrates, amino acids, and the two inorganic elements calcium and phosphorus, and makes a complex which increases the molecular weight of this complex thus making it more stable to reduce the hyper-cholestremia (Potter et al., 1993).

Another compound, hypericum contains two principle active constituents namely, hypericin and hyperforin. Hypericin is a naphthodianthrene, a red-coloured anthraquinone-derivative and is a potent photosensitizing agent exhibiting antiviral, antibacterial, antineoplastic activities. Mammalian eye lens contains a significant protein alpha-crystallins, which is a member of the small heat-shock protein family, possessing chaperone-like function. Partial unfolding of this protein increases exposed hydrophobic tryptophan, which plays a significant role in its interaction with hypericin. Partial perturbation of protein enhance the binding between alpha-crystallin and hypericin and provide a way for drug binding (Youssef, 2009).

Paeoniflorin is a phenolic substituent derived from Paeonia lactiflora, with anti-androgenic properties. As mentioned previously in this section about heat shock proteins that are induced by various stresses, and function as molecular chaperones, in addition to this, they regulate protein biogenesis and act as lifeguards against proteotoxic stresses. Treatment of cells with paeoniflorin showed enhanced phosphorylation as well as the acquisition of deoxyribonucleic acid-binding ability of heat shock transcription factor 1 (HSF1), and formation of characteristic HSF1 granules inside the nucleus. This suggests that the
### Effect of the herbal active compound on the stability and expression of the protein associated with Neurodegenerative diseases.

| S.N. | Plant                  | Active molecule                  | Method of extraction | Target protein | Function | Effect on protein | Reference                                                                 |
|------|------------------------|----------------------------------|----------------------|---------------|----------|------------------|---------------------------------------------------------------------------|
| 1.   | Rhizoma Alpiniae       | Anthraquinone N/A                | Purchased from Aladdin-Reagents | Insulin       | Stabilize     | Amyloid inhibitors | (Gong et al., 2014)                                                       |
| 2.   | Curcuma longa          | Curcumin                         | Ethanolic extract    | Presenilin 1  | Downregulation| Downregulate presenilin 1 by proteasome mediated degradation of Stat3 and prevent amyloid production | (Yoshida et al., 2011)                                                   |
| 3.   | Eucalyptus globulus    | Eucalyptol-ester                 | Obtained from Aldrich | Parp/Poly (ADP-ribose) polymerase (PARP) | Stabilize     | Prevent PARP proteolysis hence decrease BAX/Bcl-2 ratio and inhibit apoptosis | (McRae and Kennedy, 2011)                                                |
| 4.   | Gastrodia elata        | Vanillyl alcohol                 | Obtained from Sigma  | Apo-1          | Stabilize     | Inhibit mitotic spindle hence prevent from mitotic progression               | (Zhou et al., 2007).                                                      |
| 5.   | Scutellaria Baicalensis| Baicalin                          | Obtained from Aldrich | alpha-synuclein | Stabilize     | Prevent PARP proteolysis hence decrease BAX/Bcl-2 ratio and inhibit apoptosis | (Dearlove et al., 2008)                                                   |

### Effect of plant constituents on the stability or expression of cell signaling proteins

Various diseases result from the alteration in the conformation or expression of various signaling proteins. Herbal compounds are also identified which alter the conformation or expression of these proteins (Table 4). The herbal active compounds with their effect on the signaling proteins are summarized in Fig. 4.

Oridonin, a bitter tetracycline diterpenoid, a compound extracted from *Radobasia rubescens* have broad-spectrum anti-neoaplastic as well as anti-bacterial properties. This inhibits tumor cell proliferation and induces cancer cell death by regulating a series of transcription factors, protein kinases as well as pro- and/or anti-apoptotic proteins. It targets acute myeloid leukemia-associated fusion protein (AML1-ETO), an oncoprotein enhancing self-renewal of hematopoietic stem / progenitor cells, blocks hematopoietic differentiation. The AML1-ETO protein recruits nuclear receptor co-repressor (NCoR) histone deacetylase (HDAC) complex, inhibiting transcription of AML1 target genes. Oridonin induces apoptosis via reducing the number of viable cells and causing hydrolysis of peptide bonds of AML1-ETO fusion protein (Zhou et al., 2007).

Isoflavones comprise a class of organic compounds, often naturally occurring, related to the isoflavonoids found in seeds and sprouts of *Cicer aritinium*. These compounds target cell cycle proteins such as p53,
BAX, Bcl-2 etc. Isoflavones decreased the expression of anti-apoptotic Bcl-2 and increased the expression of Bcl-2-associated X (Bax) protein, caspase 7, caspase 9, p53, and p21 in a dose-dependent manner that promotes apoptosis (Chen, 2014).

Parthenolide, a sesquiterpene lactone, isolated from Tanacetum parthenium herb has anti-inflammatory properties and activates IκB kinase. NF-κB regulates the expression of genes involved in angiogenesis, invasion, and metastasis and is, therefore, an attractive therapeutic target. Activation of IκB kinases complex leads to the phosphorylation, ubiquitination, and degradation of the inhibitory IκB proteins, allowing NF-κB to enter the nucleus and regulate specific gene expression for cell survival (Yip-Schneider et al., 2005).

Moreover, ellagic acid (EA), a dimeric derivative of the gallic acid, is a naturally occurring dietary polyphenolic compound found in fruits and nuts as EA-glycosides conjugates or bound ellagitannins (ETs). EA targets caspase in their action. The CASP3 protein is a member of the cysteine-aspartic acid protease (caspase) family. Sequential activation of caspases plays a central role in the execution-phase of cell apoptosis. EA activates caspase 3 and cleavage of PARP (hallmarks of apoptosis) hence induces tumor cell apoptosis (Zhao et al., 2013).

Another compound i.e. chalcone, cardamonin (2’, 4’-dihydroxy-6’-methoxychalcone) affect cell growth via altering different cell signaling pathways, such as mTOR (mammalian target of rapamycin) and NF-κB. Cardamonin activates TRAIL-induced apoptosis through down-regulation of cell survival proteins and TRAIL-decoy receptor (DcR-1), as well as up-regulation of TRAIL death receptor (DRs), which is mediated via activation of ROS-mediated CCAAT/enhancer binding protein homologous protein (CHOP) (Yadav et al., 2012).

Trigoneline is an alkaloid isolated from Trigonella foenum-graecum seeds. Transcription factor, nuclear factor E2-related factor 2 (Nrf2) confers apoptosis protection in tumor cells, has an essential role in cancer development and chemo-resistance. Trigonelline efficiently decreased basal and tBHQ (t-tert-butylhydroquinone)-induced Nrf2 activity in all cell lines, an effect relying on the reduced nuclear accumulation of the Nrf2 protein. It is an efficient Nrf2 inhibitor which blocks Nrf2-dependent proteasome activity, hence causing apoptosis protection in pancreatic cancer cells (Arif et al., 2013).

Similarly, limonoids are phytochemicals classified as tetrnortriterpenes, which comprises variations of the furanolactone core structure. Apoptosis regulator BAX, also known as bcl-2-like protein 4 which act as anti- or pro-apoptotic regulators that are involved in a wide variety of cellular activities. The process of apoptosis is controlled by the complex interaction between regulatory proteins from the Bcl-2 family. Limonoids significantly inhibit the Bcl-2 protein level, while the Bax protein level was increased thereby inducing apoptosis (Patil et al., 2009).

Plumbagin or 5-hydroxy-2-methyl-1, 4-naphthoquinone is an organic compound and a kind of quinone commonly found in the carnivorous plants. This compound upregulates Bax, and result in a rapid decline in mitochondrial transmembrane potential, overexpression of apoptosis-inducing factors in the cytosol, cleavage of procaspase-9 and poly ADP-ribose polymerase. It downregulates phosphoinositide 3-kinase activity through a negative feedback mechanism where phosphoinisitol-3-kinases are a family of related intracellular signal transducer enzymes which phosphorylates at 3rd position hydroxyl group of the inositol ring of phosphatidylinositol (Chena et al., 2009).

Likewise, artemisinin is an active ingredient having an endoperoxide bridge structure. The compound (itself or its derivative) can inhibit human tumor cell lines growth but have selective cytotoxicity. Dihydroartemisinin (DHA), most potent among different artemisinin derivatives, has better water solubility, as well as best anti-malarial activity as compared to its parent compound. Caspases belong to a

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**Fig. 2.** Effect of plant constituents on the stability and expression of proteins associated with neurodegenerative diseases.
family of cysteine proteases involved in tumor signaling and can promote apoptosis. DHA can upregulate pro-apoptotic proteins, which finally activate caspases, hence promote apoptosis of osteosarcoma cells (Ji et al., 2011).

Studies also demonstrate that Withaferin A from *Withania somnifera* (also known as withanolides) exhibit various therapeutic activities such as neuronal regeneration and anti-herpetic property. Unlike higher eukaryotes, withaferin A can induce apoptosis in leishmanial cells by targeting its protein kinase. Protein kinases involved in the gene activation, cell differentiation and release of neurotransmitters. Bioinformatics study stated that withaferin A bind to leishmanial protein kinase-C (LPKC) thereafter inhibit leishmania infection (Grover et al., 2012).

Acacetin (5,7-dihydroxy-4’-methoxyflavone) is a flavonoid compound which targets MAPK, which are the mitogen-activated protein kinases super-family members associated with increased scattering/motility, invasion, proliferation, survival, and morphogenesis. The molecule inhibits phosphorylation of p38 mitogen-activated protein kinase (p38 MAPK), which is involved in the down-regulation of matrix metallo-proteinase-2 (MMP-2), matrix metalloproteinase-9 (MMP-9), and urokinase-type plasminogen activator (u-PA) at both the protein and mRNA levels, which finally inhibit cancer cell metastasis (Hsu et al., 2004).

Furostanol (saponins) were recognized as major saponin of the *Allium* genus. PTPIB (protein tyrosine phosphatase 1B) serves as a key negative regulator of the tyrosine phosphorylation cascade integral to the insulin-signaling pathway. Results of 1D and 2D NMR showing 81.1% inhibition of PTPIB with the cepharoids E-L which are the potent inhibitors of PTPIB and cytotoxic activity against lung adeno-carcinoma (A-549) and improve the functions of internal organs, treat diarrhea, and puffiness of the face and eyes, as well as promote blood flow (Li et al., 2014). An oxazole-based peptidomimetic of Stat3 Src homology 2 domain-binding phosphoryrosine peptide is S3I-M2001, which disrupts active Stat3:Stat3 dimers selectively. Stat3 activity involves in dysregulated growth and survival, angiogenesis and suppresses host’s immune surveillance of tumor. Stat3 is a very important molecular mediator of carcinogenesis as well as tumor progression. Inhibition of Stat3 activation and its dimerization was observed in the presence of S3I-M2001 that has significant antitumor activity (Siddique et al., 2007).

Another molecule, luteolin (3, 4, 5, 7-tetrahydroxyflavone) is a natural flavonoid, which suppresses UVB-induced cyclooxygenase-2 expression, activator protein-1, and nuclear factor κB activity. Protein kinase Ce (PKCe) is a calcium-independent PKC isoform linked to proliferation, differentiation, and carcinogenesis in the skin. Luteolin attenuated protein kinase Ce (PKCe) and Src kinase activities, subsequently inhibit UVB-induced phosphorylation of mitogen-activated protein kinases and Akt signaling pathway and finally reduces skin cancer (Byun et al., 2010).

Embelin is a low molecular weight natural benzoquinone extracted from *Embelia ribes* (Radhakrishnan et al., 2011), which has been reported as potent non-peptidic, cell membrane permeable molecule and an inhibitor of XIAP (X-linked IAP). The inhibitors of apoptosis proteins (IAPs) were recently discovered as an important class of intrinsic cellular inhibitors of apoptosis. Intrinsic and extrinsic apoptosis pathways are inhibited by XIAP protein. Embelin interacts with numerous basic residues in the BIR3 domain of XIAP where Smac and caspase-9 bind. Cell growth in cancer cells with high levels of XIAP protein is selectively inhibited by embelin, which has less activity in the normal cells with low levels of XIAP proteins (Radhakrishnan et al., 2011).

In brief, the specific signaling pathway governs every metabolic activity. Alteration in the proteins involved in signaling pathway can cause lethal diseases. For proper functioning of cancer signaling, different chemotherapeutics drugs are prescribed but sometimes they may not work correctly. Therefore, these herbal compounds can be explored to develop a therapeutics targeted to cell signaling.
Effect of phytoconstituents on the stability and expression of viral proteins

Plant active compounds are also used as an antiviral compound. Some of these herbal active compounds (Table 5) are discussed below.

Hypericin, an anthraquinone dimer known to have activity against non-human retroviruses also exhibited anti-HIV-1 activity in lymphocytes. The active anthraquinones inhibit HIV-1 reverse transcriptase. Reverse transcriptase (RT) used to generate complementary DNA from an RNA template. RT is needed for replication of retroviruses (e.g., HIV), and RT inhibitors are widely used as antiretroviral drugs. However, this enzyme inhibition was selective only for 1, 2, 5, 8-tetrahydroanthraquinone and hypericin. Hypericin interacts non-specifically with RT and inhibits it. Assembly or processing of intact virions from infected cells was affected by hypericin. Hypericin-treated, virus-producing cells produce particles containing immature or abnormally assembled cores, which suggest that it may interfere with processing of gag-encoded precursor polyproteins. The released virions also contain no detectable activity of reverse transcriptase (Schinazi et al., 1990).

Some other reports demonstrate emodin as an anthraquinone compound derived from genus *Rheum* and *Polygonum* and consists of three cyclic rings. SARS-CoV spike protein, a type I membrane glycoprotein, on viral envelope requires for fusion of virus to the host cell receptor angiotensin-converting enzyme. Emodin disrupts the protein interaction with host cell receptor angiotensin conversion which prevents the viral attachment to host thus protecting from severe acute respiratory syndrome (Ho et al., 2007).

Coumestrol (isoflavonoid) is reported to possess the antixenosic properties. Breast cancer resistance protein (BCRP) is an ATP-binding cassette transporter, shown to confer multidrug resistance (MDR) to a number of anticancer agents and play an important function in governing drug disposition. BCRP restricts the absorption of orally administered drugs. Isoflavonoids coumestrol strongly inhibited BCRP, and its inhibitory potencies are due to its binding to the functional moieties at C5 position which inhibit the BCRP and leads to the availability of chemotherapeutic drugs (Tamaki et al., 2010).

The therapeutic efficacy of herbal active compounds at low doses propose the necessity for investigating if the resultant effects are due to multi-ingredient synergistic effects or placebo. 2,4,6-trinitrobenzene sulfonic acid causes ulcerative colitis in male Wistar/ST rats. The saponin fraction of ginsenosides and glycyrrhizin were individually ineffective but exhibited the synergistic effect when used in combination for reducing ulcerative colitis. Combination effectively reduces defective responses during ulcerative colitis model involving both Th1 and Th2 responses. Similarly, 1,25-dihydroxy vitamin D3 (VD) exhibits potent pro-differentiative, anti-proliferative and immunomodulatory activities by antagonizing vitamin D receptor. Lycopene inhibits cell cycle progression via the retention of p27 in cyclin E–cdk2 and reduction of the cyclin D level. The synergistic effect of low concentration VD and lycopene reduces cell proliferation and differentiation in pro-myelocytic leukemia cell line. Upregulation of cyclin D via Vitamin D receptor counters the reduction of cyclin D by lycopene. Hence the vitamin D receptor antagonism due to VD, reducing the counteractive action, which has resulted in pharmacodynamic synergism (Ma et al., 2009).

In brief, viruses are responsible for causing many severe infectious diseases, and hence it is essential to take the precautions for viral infections. It is a very tough task to fight against viral infection via drug formulation. Some herbal compounds have the potential to act as an alternative to cure diseases such as hypericin, inhibiting the viral reverse transcriptase and thus can be developed as an important drug against HIV.

Conclusion and future prospects

With increasing population, the probability of the occurrence of diseases also increases. To control this situation, it is necessary to find novel drugs for different disease or pathogens. Different geographical regions have different plants species with diverse potential active compounds, which are immensely used as traditional medicines. The major target of the herbal compound is found to be proteins such as enzymes and transporters. These active compounds can also bind or
### Table 4. Effect of the herbal active compound on the stability and expression of the proteins associated with cell signaling.

| S.No. | Plant                          | Active molecule                  | Method of extraction | Identification | Target protein | Effect on protein | Function | Reference                                      |
|-------|-------------------------------|----------------------------------|----------------------|----------------|----------------|-------------------|----------|-----------------------------------------------|
| 1     | Rabdosiarubescens             | Oridonin (tetracycline diterpenoid compound) | N/A                   | NA             | acute myeloid leukemia-associated fusion protein (AML1/ETO) | Destabilize | Hydrolysis of peptide bond of AML1/ETO protein and Induce apoptosis | (Zhou et al., 2007) |
| 2     | Cicer aritinum                | Isoflavones                       | N/A                   | (Annexin V/PI) staining | Cell cycle protein as Bax, p53, Caspases | Upregulation | Increase apoptotic body formation by decreasing BCL-2 and increase Bax, p53 and caspases | (Chen H, 2014) |
| 3     | Tanacetumparthenium(feverfew) | Parthenolide and sulindac         | N/A (Purchase from sigma) | NA             | Bcl-2 kinase | Stabilize | Activation of Nfkβ kinase complex leads to the NF-kβ to enter in nucleus | (Yip-Schneider et al., 2005) |
| 4     | Caryallinoineinis             | Ellagic acid (EA) polyphenol      | purchased from LKT Laboratories | Western blot | Caspase protein (CASP-3) | Stabilize | Cleavage of substrate PARP, substrate of Caspase, leads to apoptosis | (Zha et al., 2013) |
| 5     | BetariaCardamomumMaton        | Chalcone cardamom                 | from Tocris Bioscience, Ellisville, MO, USA | NA             | TRAIL Death receptor | Upregulation | Enhance apoptosis | (Yadav et al., 2012) |
| 6     | Trigonella foemungreaeum      | Trigonelline (alkaloid)           | NA                   | Nrf2 transcription factor | Destabilize | Degradation of Nrf 2 promote apoptosis | (Arif et al., 2013) |
| 7     | Lime (Citrus aurantifoliaSwingle) | Limonoid                          | MeOH extract/ (HPLC)  | NA             | Bax, (Apoptosis regulator BAX) | Up-regulation | Proliferation inhibition induce apoptosis | (Putl et al., 2009) |
| 8     | Plumbago seylanica            | Plumbagin                         | N/A                   | Liu's staining and transmission electron microscopy western blot | phosphoinositide 3-kinase | Down-regulation | Downregulate phosphoinositide 3-kinase activity via negative feedback mechanism and induce apoptosis | (Chena et al., 2009) |
| 9     | Artemisia annua               | Dihydroartemisinin                | N/A (Obained from Sigma Aldrich) | Caspase-3 | Upregulation | Activate cell cycle protein, induction of apoptosis and cell cycle arrest. | (Ji et al, 2011). |
| 10    | Withania somnifera            | Withaferin A                      | NA                   | Leishmanial protein kinase C (LPKC) | Destabilize | Inhibit LPKC induce apoptosis | (Grover et al., 2012) |
| 11    | Cirrhisium rhinoceros         | Acacetin (flavonoid)              | N/A (Purchase from sigma) | NA             | mitogen-activated protein kinases | Downregulation | Inhibit cancer cell metastasis | (Hsu et al., 2004). |
| 12    | Allium cepa L                 | Furostanol (saponins)             | n-butanol fraction of ethanol extract /HPLC | 2D NMR | Protein tyrosine phosphatase IB (PTP1B) | Destabilize | Inhibit PTP1B and show cytotoxic activity for human cell line | (Li et al., 2014) |
| 13    | Allium cepa, Brassicaoleracea,ApiumGraveolens L | Luteolin (purchased from Indofine Chemical Company) | NA | Protein Kinase C-e | Destabilize | Activate PKC by dephosphorylation and reduce skin cancer | (Byun et al., 2010) |
block the site responsible for binding of the pathogenic element. These plant-derived therapeutics can also alter the mechanism of action or conformation of biomolecules or can change their expression for disease-causing state. We can also identify their target and mode of action by using advance approaches so that the possibility of disease-causing protein conformation can be reduced in future. Metabolic engineering of plants for increased biosynthesis of antimicrobial compounds may open a new way out for discovery of novel therapeutic agents. Therefore, in the present review, we have listed plants active compounds that alter conformation or expression of disease-causing proteins; hence they could be useful for producing a novel drug to cure diseases. The detailed biochemical and biophysics study is required to develop phytoconstituents as therapeutics targeting to protein conformation. Phytoconstituents need to be checked for its ADMET properties (Tian et al., 2015), pharmacophore mapping (Tian et al., 2013), efficacy and safety before used as a therapeutics.

Conflict of interest

We wish to confirm that there are no known conflicts of interest associated with this publication.

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Supplementary materials

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Table 5.

| S.No | Plant            | Active molecule | Method of extraction | Identification | Target protein | Effect on protein | Function | Reference                        |
|------|-----------------|-----------------|----------------------|----------------|-----------------|-------------------|----------|----------------------------------|
| 1    | *Artemisia annua* | Hypericin       | NA                   | NA             | HIV-1 reverse transcriptase (RT) | Destabilize       | Release virion thus loss activity of RT | (Schinazi et al., 1990) |
| 2    | *Rheum emodi*   | Emodin          | N/A (Purchased from sigma) | NA             | SARS-Co spike protein | Destabilize       | Inhibit the interaction of S protein and ACE2 receptor | (Ho et al., 2007) |
| 3    | *Glycine max*   | Coumestrol (Isoflavonoid) | Hydrous ethanol | Purchase from sigma | BCRP (breast cancer resistance protein) | Destabilize  | Increase system availability and its substrate drug uptake | (Tamaki et al., 2010) |

Fig. 4.. Effect of herbal compounds on the stability of cell signalling proteins.

Table 5.

Effect of the herbal compound on the stability and expression of other proteins.
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