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Green Synthesis, Biological Analysis and *in silico* Studies of Biosynthesized Silver Nanoparticles from Ethanolic Extract of a Medicinal Herb, *Kaempferia galanga* as Anti-Inflammatory Agent

Dowluru S.V.G.K. Kaladhar and Barsha Mohanty

Department of Microbiology and Bioinformatics, UTD,
Atal Bihari Vajpayee University, Bilaspur (CG), India

Abstract: The silver nanoparticles act as carrier and also activator for many drugs to target diseased proteins. The present experimentation was conducted on MyD88 and IRAK-4 inhibitors as anti-inflammatory agent in *E. coli*. The AgNP of ethanolic extract of *Kaempferia galanga* L. rhizome increases the antimicrobial activity (25mm zone of inhibition) compared to chemical ethanolic extract of *K. galanga* rhizome extract (12 mm zone of inhibition). The formation of clumps between ethanolic extract of *K. galanga* and albumin is more followed by penicillin and AgNP ethanolic extract of *K.galanga*. The AgNP-ethanolic extract of *K.galanga* is degraded gelatin on X-ray film with proteins like albumin and Hemoglobin. The MyD88 protein has shown good interaction with IRAK4 in inflammation pathway based on string database. The application of AgNP synthesis of plant compounds provided more activity compared to ancient chemical process. The electrostatic interaction of (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid with IRAK4 decreased the activity (-74.2 Kcal/mol) compared to non-electrostatic interaction of AgNP- (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid with IRAK4 (-98.1 Kcal/mol). Hence AgNP of plant extract interactions removes electrostatic interactions and increases the activity on mutated proteins like IRAK4 that causes inflammation.

Key words: AgNP · Green Synthesis · *In silico* studies · *Kaempferia galanga*

INTRODUCTION

The synthesis of nanoparticles is an eco-friendly technique that applies the usage of biological agents like plants or microbes are been using as pharmaceutical products [1, 2]. The medicinal plants under certain controlled conditions are possible to synthesize nanoparticles with desired properties. The green synthesized nanoparticles provide enhanced antimicrobial activity in comparison with chemically synthesized nanoparticles [3]. The particles act as capping and reducing agents by green chemistry offers a novel and potential alternative using silver nanoparticles. Researchers use bionanotechnology techniques that are being applied for various purposes in pharmaceutical and industrial applications [4, 5].

Plants can produce a variety of phytocompounds to protect themselves against several disease causing pathogens, like *Staphylococcus aureus* and *Escherichia coli* [6, 7]. Biological molecules like plant drugs target receptors using silver, copper, gold etc., as a carrier and active elements [8]. A receptor inhibitor is a type of ligand, protein or drug that blocks a biological response by binding to a receptor and makes reduction of the stimulation of the receptor by its ligand [9]. The core takinib aminobenzimidazole molecule is intrinsically acted towards Transforming growth factor beta-activated kinase 1 (TAK1) which is closely related interleukin-1 receptor-associated kinase (IRAK) family members and can be effectively used as super-selective inhibitors [10]. In this direction biosynthesized Silver Nanoparticles (AgNPs) of ethanolic rhizome extract from *K. galanga* is analyzed as anti-inflammatory agents in the present study.

Mechanism of MYD88 and IRAK4 in *E.coli* in Inflammation: *E. coli* (Escherichia coli), is a gram-negative, non-endospore forming, rod-shaped, facultative anaerobic, coliform bacterium that are found in the

Corresponding Author: Dowluru S.V.G.K. Kaladhar, Department of Microbiology and Bioinformatics, UTD, Atal Bihari Vajpayee University, Bilaspur (CG), India.

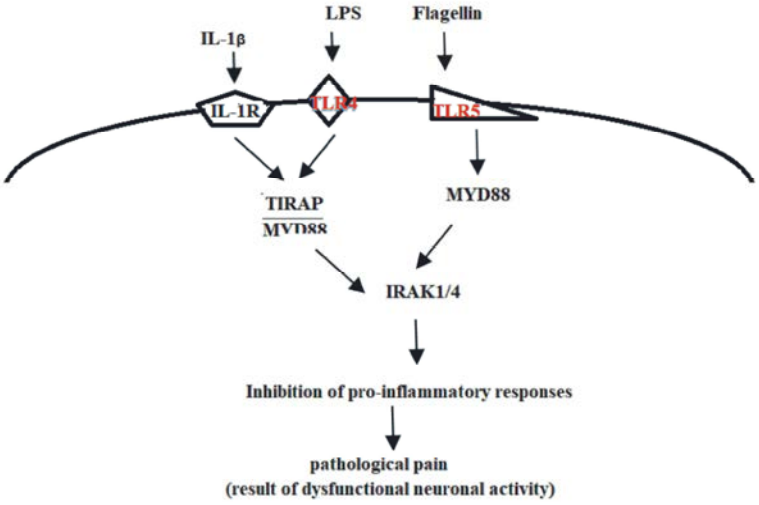

Fig. 1: *E Coli* Pathogenic infection pathway

intestines of humans and animals, used as a model fecal indicator [11]. Blocking IL-1 or TNF-α through Myeloid Differentiation Primary Response protein (MyD88) and IRAK4 is been highly successful in controlling inflammation in patients with Graft-versus-Host Disease (GvHD), rheumatoid arthritis, or inflammatory bowel disease [12,13]. The pathogenic proteins in *E. coli* like MyD88 and IARK4 causes inhibition of pro-inflammatory responses. A pro-inflammatory response is a part of natural immune defense, an undeclined pro-inflammatory cascade with excessive generation of Reactive oxygen species (ROS) for a longer duration leads to cellular injury [14]. Patients with MyD88 and IRAK4 with genetic defects are phenocopies are different with normal immune phenotype [15].

Figure 1 shows that MyD88 links IL-1 receptor (IL-1R) or TLR family members activates IL-1R-associated kinase (IRAK) family kinases via homotypic protein-protein interaction. Several inactivating mutations in MyD88 genes in humans have been identified leading to frequent infections with pyogenic bacteria. These mutations are associated with rare missense polymorphisms leads to reduced IRAK4 activation and impaired responses through IL-1 and Toll-like receptors (TLRs) family members [16,17]. MyD88-deficiency in humans doesn't appear abnormally are susceptible to many viral, fungal, or parasitic infections. Autosomal recessive IRAK-4 and MyD88 deficiencies impair TLRs and IL-1 receptor-mediated immunity is recently described as primary immunodeficiencies [18].

Autosomal recessive IRAK-4 and MyD88 deficiencies mostly susceptible to invasive bacterial infections, mostly by gram-positive bacteria like Streptococcus pneumoniae and Staphylococcus aureus and rarely by gram-negative bacteria like Pseudomonas aeruginosa and Shigella sonne [18,19]. Patients with IRAK-4 deficiency mostly have the recurrent invasive infections like cellulitis, meningitis, sepsis and osteomyelitis. A unique feature of the diseases is due to lack of inflammatory response with elevated erythrocyte sedimentation rate **(**ESR) and C-reactive protein (CRP), fevers and despite severe infections due to the damaging process to upregulate pro-inflammatory cytokines.

The innate immune system in living beings especially animals is designed to provide immediate protection from infection. The process is similar to an adaptive immune system that requires time to mount with a specific response to develop immunity. Toll-like receptors (TLRs) are some of the examples of these pattern recognition receptors. MyD88 and IRAK-4 are greatly essential for all TLR signaling except for the pathway of TLR3 [20]. Compound heterozygous or homozygous mutations in IRAK-4 cause the deficiency of normal IRAK-4 proteins result in a lack of protein expression. Vaccination with protein-conjugate and polysaccharide vaccines can be initiated as therapy of the control of IRAK4 deficiency diseases like inflammation.

Green Synthesis: Green synthesis is a method of nanomaterials from plants that is rapidly growing in the nanotechnology field which replaces the use of toxic chemicals and time consumption [21, 22]. The green synthesized silver nanoparticles can be used in the field of medicine, due to their high antibacterial activity. A process of preparing natural source as starting material may be in the form of nanopowder is a nano metal or nano

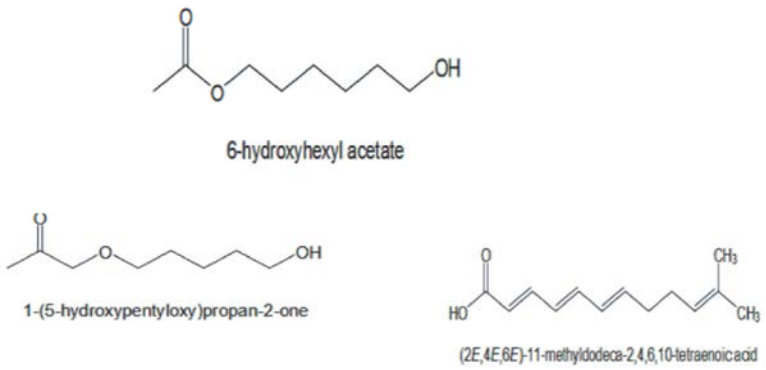

Fig. 2: Compounds present in *Kaempferia galanga* proposed by Narasinga and Kaladhar

alloy or nano composite or nano metal carbide or nano metal oxide or nano compound or nanofluid. The nano product produced has novel properties like enhanced hardness, thermal properties, antibacterial properties, electrical properties, wear resistant, sliding wear resistance, abrasive resistant, superior frictional properties, enhanced tensile strength, enhanced load bearing capacity, compression strengths and corrosion properties [23, 24]. The nano products produced are usable and used in preparation of anti-fungal/bacterial/fouling coatings, thermal fluids, paints, high corrosion resistant coatings, high strength electrical conductors, high strength electrical alloys, inkjet inks, neutralizing gram negative bacteria, neutralizing gram positive bacteria, neutralizing viruses, motor cycle clutch, rocker arm, solder materials, bearing applications, automobile parts, spring materials, steering wheel joints and coatings, connecting rod, hard disks, pen drives, memory enhancing devices, electronic chips, smart materials, shape memory alloys, add-on materials for composite lamina or laminates, etc.

Kaempferia Galanga: Kaempferia galanga L. (Family *Zingiberaceae*) is an endangered medicinal plant with potent medicinal activities [25, 26]. Aromatic ginger, Kaempferia galanga L, is a native to India and believed to be originated in Burma. The succinylation of native starch isolated from the rhizomes of *Kaempferia galanga*, is resilient material in sustained-drug release systems. Indigenous medical practitioners use these rhizomes for treatment of psoriasis, bacterial infections and tumor.

Kaempferia galanga, commonly known as kencur or aromatic ginger, is present in the countries like Indonesia, Taiwan, southern part of China, Cambodia and India [27]. The dried rhizome powder of *Kaempferia galanga* is used in herbal medicine contains 3-carene, cineol, borneol, ethyl cinnamate, camphene, kaempferol, cinnamaldehyde, kaempferide, p-methoxycinnamic acid and ethyl p-methoxycinnamate. The ethanolic rhizome extract of K.galanga is also containing compounds like (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid [28], 1-(5-Hydroxypentyloxy) Propan-2-One [28] and 6-hydroxyhexylacetate [28] has been reported by Narasinga and Kaladhar, 2019 (Figure 2). The Extracts of dried rhizome powder from *Kaempferia galanga* is used as an insect repellent that kill larvae of several species of mosquito and other insects. Dried rhizome powder of *Kaempferia galanga* is a bitter and stimulant herb used as antibacterial, treat colds, bronchial complaints, dyspepsia, gastric complaints, headaches, treatment of high blood pressure, wounds, treat dandruff, swellings, ulcers, asthma, rheumatic joints, improves the digestion and has diuretic effect.

Role of Starch on MYD88: The salivary digestive enzymes are vital in the processing of dietary proteins, fats and starches [29]. Antimicrobial agents are also contained in saliva and some products on tongue, regularly protecting the surfaces of oral mucosa from microbial infections [30]. MyD88 plays a basic role in *in vitro* and *in vivo* ras-dependent carcinogenesis. Solid compositions for oral administration like tablets, powders (gelatin capsules, sachets), pills or granules have the active ingredient mixed with one or more inert diluents, like starch, sucrose, lactose, cellulose or silica, under an argon stream. Dietary prebiotics, like resistant starches provides an alternative to antibiotics for improved animal health, including humans. Important bacteria that consume resistant starches are normal members of the human microbiota are associated with intestinal health [31].

(A) Aqueous extract of KG (B) AgNP Aqueous extract of KG (C)Aqueous solution (D)Ethanolic extract of KG (E) AgNP Ethanolic extract of KG

Fig. 3: Samples prepared for the present experimentation

MATERIALS AND METHODS

Preparation of Plant Extract: The dried sample of *K.galanga* rhizome was made powder using motor and pestle. The powder *K.galanga* is stored in refrigerator for further exaction and experimentation. About one gram of powder of *K.galanga* rhizome was mixed with 10 ml of double distilled water and prepared aqueous extract of *K.galanga* rhizome. The extract was filtered using Whatman filter paper Grade 1 and the filtrate was purified further using centrifugation at 4000 rpm for 20 minutes (concentration aqueous extract of *K.galanga* rhizome is 100 mg/ml). The filterate was dried to powder by keeping filterate in sunlight in a protected and covered petriplate. About one gram of powder was mixed in 10 ml of ethanol and prepared ethanolic extract of *K.galanga* rhizome. The Ethanolic rhizome extract of *K.galanga* was separated using centrifugation at 4000 rpm for 20 minutes for getting better pure sample (concentration of Ethanolic rhizome extract of *K.galanga* is 100 mg/ml) [32].

Biosynthesis and Characterization of Silver Nanoparticles: About one gram of powder was mixed in 10 ml of ethanol was prepared. 5 ml of the extract was added to 100ml of $1X10^{-3}$ M aqueous silver nitrate solution at room temperature. The solution was kept in sunlight for 3 hours in summer season (around 47 degree centigrade). The yellow color of solution changes to black suspension mixture after 3 hours. The AgNP from ethanolic extract of *K.galanga* was separated using centrifugation at 4000 rpm for 20 minutes (concentration AgNP of ethanolic extract of *K.galanga* is 100 mg/ml). The extracts of AgNP, ethanolic and aqueous are shown in Figure 3. The final AgNP, of ethanolic, control and aqueous extracts was centrifuged at 6000 rpm for 15 minutes and the supernatant was selected in present experimentation [32].

Characterization of particles is implemented by calorimetric method. The OD was recorded at different wave lengths and the time.

Slide method and Microscopy of Biosynthesized AgNPs: The extracts are mixed with proteins like albumin and hemoglobin respectively and were observed for formation of coagulation on glass slide. The sample was observed under optical microscope at 100X magnification [32].

Enzyme Hydrolysis by X-ray Film Method of Biosynthesized AgNPs: Approximately 10 µl of protein activator (AgNP extracts of *K. galanga*) was mixed with 10 µl of protein (Albumin or Hemoglobin of 0.5 mg/ml) and was spotted onto a stripe of the X-ray film. 10 µl of Penicillin was mixed with 10 µl of 0.l M (pH 7.0) phosphate buffer as the control and was spotted on to the X-ray film. The above inhibitor and protein mixtures were incubated of X-ray film at 37°C for 5 minutes. After 5 minutes, wash the film under tap water gently without touching X-ray film with hands or other objects for the clear observation of zone of gelatin hydrolysis. The non-protein activity will be visualized as thick color without gelatin hydrolysis and protein activity will be shown as zone of gelatin hydrolysis [32].

Antibacterial Activity of Biosynthesized Ag Nps: Antibacterial activity test was carried out by well diffusion method. The 10 mm wells on *E. coli* inoculated agar plates were filled with 10 µl of the Control (sterile double distilled water), standard (Penicillin), $AgNO_3$ solution, ethanol extract of *K. galanga* and AgNP ethanolic extract of *K. galanga* at the concentration of 100 mg/well. Sterile distilled water was added as control. Penicillin (Antibiotic) was used (100 mg/well) as positive reference standard. The Muller Hinton agar plates were incubated at 37°C for 24 hours. The inhibitory activities of the samples were quantitatively assessed by observing the presence or absence of the inhibition zones and the zone diameters (including well size of 10 mm). The AgNPs have excellent antimicrobial property may be due to their extremely large surface area [32, 33].

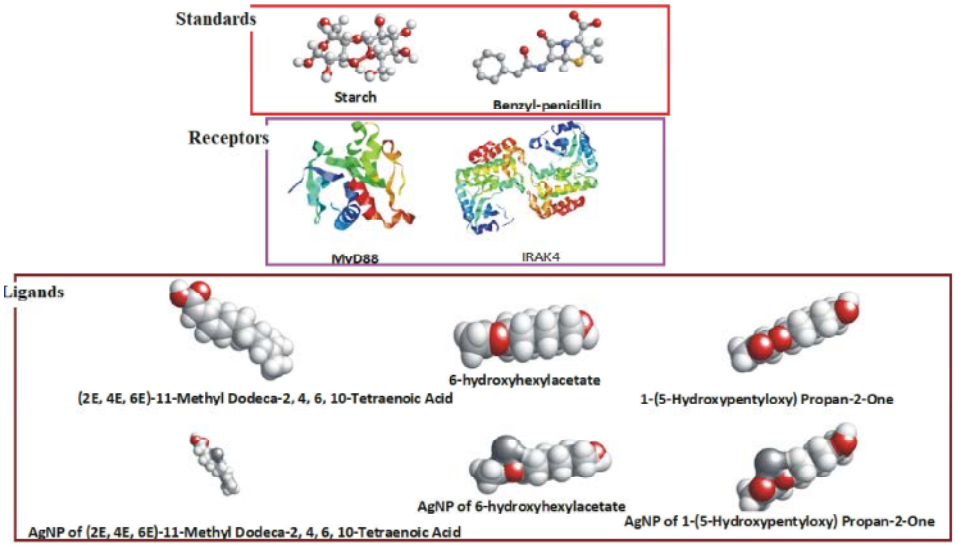

Fig 3. Standards, Ligands and Receptors selected in the present study

***In silico* Analysis:** The ligands are designed using Chemsketch software of ACD/Labs v10.02. The receptor (MyD88 with pdb id 4DOM; IRAK4 with pdb id 6RFI) is retrieved from PDB database. Both the molecules are docked using iGEMDOCK v2.1 for screening better compounds from the selected ligands as antimicrobial agents [32] (Figure 3).

RESULTS AND DISCUSSION

Calorimetric Analysis: Calorimetric absorption spectrum of AgNPs is shown in Figure 4. Maximum peak of silver occurs at 450 to 540nm. This peak increased with time. The intensities are frequently used to determine the global binding mechanisms of protein-ligand or protein-protein interactions. Room temperature was used for colorimetric intensity monitoring in the absorption spectra changes. The number of peaks increases by increasing diversity of particles shapes (Figure 4 and 5). The brown supernatant is considered as nanoparticles as per the experimentations done in previous performed literature and difference in optical densities in calorimeter.

The optical microscopic examination of Albumin, Penicillin interaction with Albumin interaction and AgNP of ethanolic extract of *K.galanga* interaction with albumin is shown in Figure 6. The albumin has fibre like observation under microscopic observation. The penicillin was reacted with albumin and formed clumps. The AgNP of ethanolic extrct of *K.galanga* interaction with albumin didn't shown clumps.

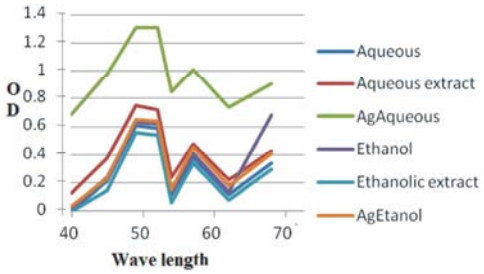

Fig. 4: Calorimetric analysis of aqueous and ethanol extracts and solvents with wave length

The interaction was further analysed on glass slide (Figure 7). The formation of clumps between ethanolic extract of *K.galanga* and albumin is more followed by penicillin. There is less reaction with AgNP ethanolic extract of *K.galanga* with albumin. The aqueous extract of *K.galanga* is dissolved with albumin.

The antimicrobial activity of ethanolic extract and AgNP ethanolic extracts of *K.galanga* is compared with penicillin as standard (Table 1). The standard (Penicillin) at 100mg concentration found good zone with *E. coli* (44mm zone). AgNP of ethanolic extract of *K.galanga* at 100mg got less zone of inhibition (25mm zone) compared to standard. The zone of inhibition is also found in ethanolic extract of *K. galanga* (12mm zone). The report shows that AgNP increases the antimicrobial activity compared to chemical extracts. The pure compounds of extracts of *K.galanga* may provide better antimicrobial activity results.

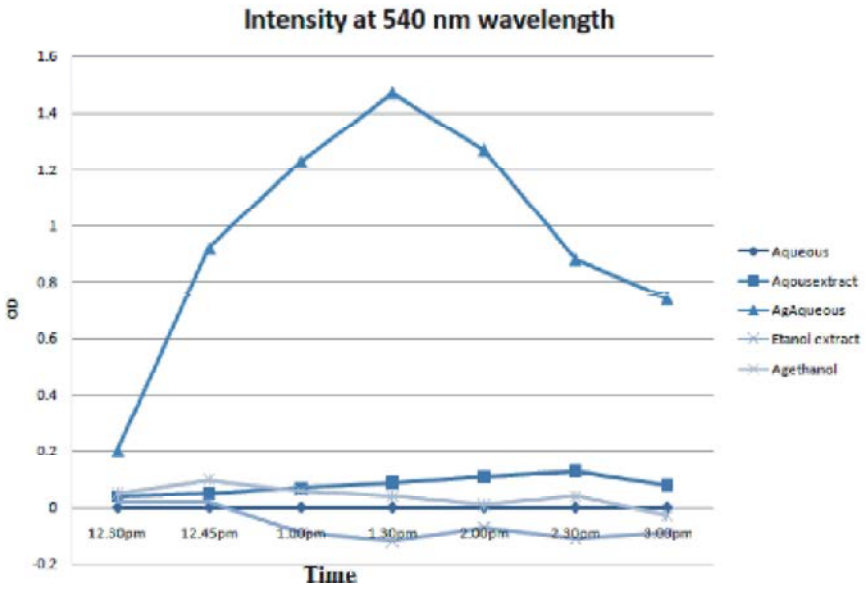

Fig. 5: Calorimetric analysis of aqueous and ethanol extracts with time at 540nm wavelength

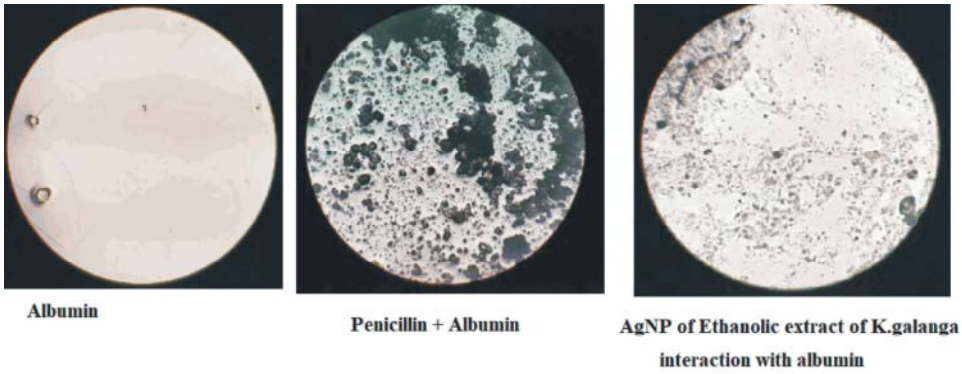

Fig. 6: Microscopic examination

Fig. 7: Microscopic Slide method for understanding *K.galanga* extract interaction with albumin

Table 1: Antimicrobial activity of biosynthesized AgNPs of ethanolic rhizome extract from *K. galanga*

Extract	Zone of inhibition
Control-Sterile distilled water	0
$AgNO_3$ solution	0
Standard	44 mm
Ethanol extract	12 mm
AgNP-Ethanolic extract of *K.galanga*	25 mm

Figure 8 and Table 2 has shown that AgNP-ethanolic extract of *K.galanga* (KG) is degraded gelatin on X-ray film with proteins like albumin and Hemoglobin. The aqueous extract and ethanolic extract with albumin has shown gelatin degradation (shown good interaction). The aqueous extract and ethanolic extract with hemoglobin has not shown gelatin degradation (no interaction).

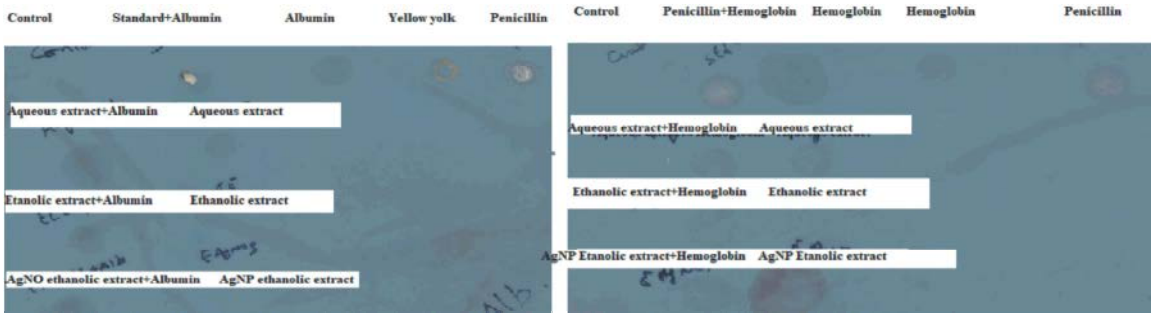

Fig. 8: X-ray film method for gelatin degradation

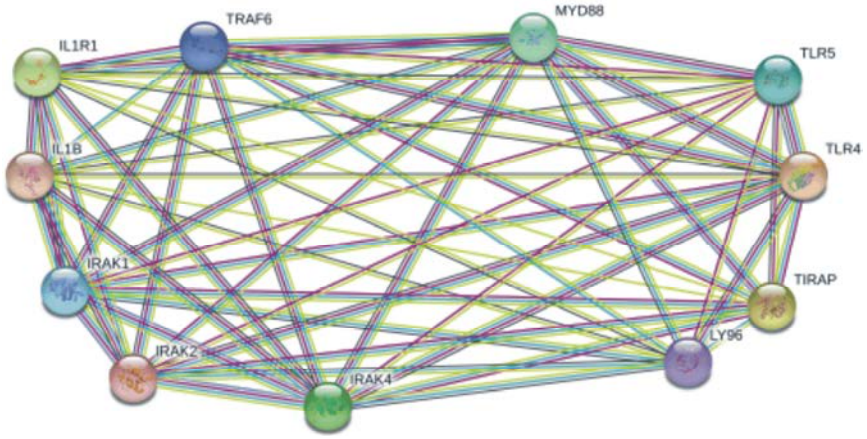

Fig. 9: Protein-Protein interaction analysis for IRAK4 and MyD88 using string server

Table 2: X-ray film method for gelatin degradation

Control	Aqueous	Albumin	Hemoglobin
Aqueous extract of KG	+	++	+
Ethanol extract of KG	-	+	-
AgNP Ethanolic extract of KG	-	+++	+
Control	-	+	++
Standard	+	++	++
Yellow egg yolk	++	NA	NA

Figure 9 shows the protein-protein interaction analysis using string protein interaction server. The MyD88 protein has shown good interaction with IRAK4 in inflammation pathway.

Table 3 and 4 shown that (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid, 6-hydroxyhexylacetate, 1-(5-Hydroxypentyloxy) Propan-2-One, Benzylpenicillin, AgNP-(2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid, AgNP-6-hydroxyhexylacetate, AgNP-1-(5-Hydroxypentyloxy) Propan-2-One and Starch are better anti-inflammatory agents that control pathogenic *E. coli*. The application of AgNP synthesis of plant compounds provided more activity compared to ancient chemical process. The electrostatic interaction of (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid with IRAK4 decreased the activity (-74.2 Kcal/mol) compared to non-electrostatic interaction of AgNP- (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid with IRAK4 (-98.1 Kcal/mol). Hence AgNP of plant extract interactions removes electrostatic interactions and increases the activity on mutated proteins like IRAK4 that causes inflammation (Figure 10).

Interleukin-1 receptor (IL-1R)-associated kinase-4 (IRAK-4) plays a key role in alleviating chronic inflammatory and autoimmune disorders. IRAK-4 belongs to a family of mammalian IRAKs that include IRAK-1, IRAK-2 and IRAK-3 [34]. IRAK-4 is considered as "master IRAK", the only family member that is essential for IL-1R/TLR signaling. IRAK-4 is a key signaling component downstream of Toll like receptor (TIR) family includes IL-1R, IL-18R and Toll-like receptors. It is possible that modulation of IRAK-4 function by kinase inhibition may provide therapeutic benefits by reducing inflammatory responses [35]. Nevertheless, current targets available did not offer sufficient relief in some patients and have a broad spectrum of adverse events in the subjects. In this regard, emphasis is laid on highlighting development of small molecule inhibitors targeting the

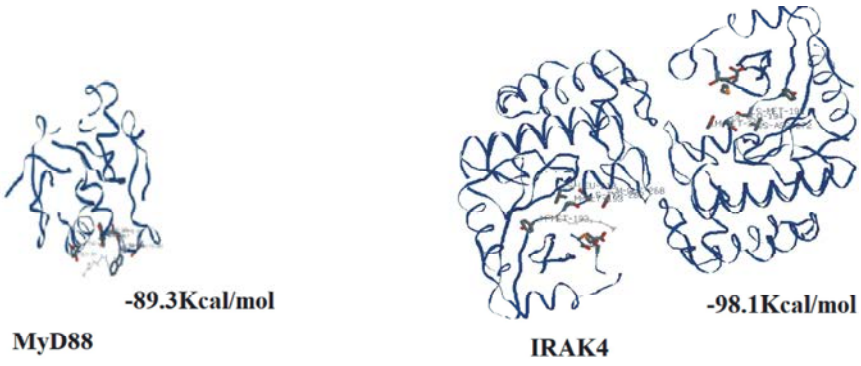

Fig. 10: Interaction of AgNP- (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid with MyD88 and IRAK4 respectively

Table 3: Interaction analysis of biosynthesized *K.galanga* compounds with MyD88

Compound	E Value	Active site
(2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid	-94.8	V-S-PHE-164-V-M-TYR-167-V-S-TYR-167-V-M-SER-194-V-S-SER-194-V-M-ASP-195-V-S-ASP-195-V-S-VAL-198-V-M-GLU-232-V-S-GLU-232-V-S-PHE-235
6-hydroxyhexylacetate	-85.1	V-S-GLN-181-V-S-TYR-276-V-M-LYS-282-V-S-LYS-282-V-M-TRP-284-V-S-TRP-284-V-M-PHE-285-V-S-PHE-285-V-S-TRP-286
1-(5-Hydroxypentyloxy) Propan-2-One	-89.7	V-S-GLN-181-V-S-TYR-276-V-M-LYS-282-V-S-LYS-282-V-M-TRP-284-V-S-TRP-284-V-M-PHE-285-V-S-PHE-285-V-M-TRP-286-V-S-TRP-286
Benzylpenicillin	-87.4	H-S-TYR-276-V-S-GLN-181-V-S-TYR-276-V-M-LYS-282-V-S-LYS-282-V-M-TRP-284-V-S-TRP-284-V-S-PHE-285-V-M-TRP-286-V-S-TRP-286
AgNP-(2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid	-89.3	V-M-GLN-184-V-M-THR-185-V-M-LYS-282-V-M-TRP-284-V-S-TRP-284-V-M-PHE-285-V-M-TRP-286-V-S-TRP-286-V-S-THR-287
AgNP-6-hydroxyhexylacetate	-88.5	V-S-TYR-276-V-M-LYS-282-V-S-LYS-282-V-M-TRP-284-V-M-PHE-285-V-S-PHE-285-V-M-TRP-286-V-S-TRP-286
AgNP-1-(5-Hydroxypentyloxy) Propan-2-One	-88.9	V-S-TYR-276-V-M-LYS-282-V-S-LYS-282-V-M-TRP-284-V-S-TRP-284-V-M-PHE-285-V-S-PHE-285-V-M-TRP-286-V-S-TRP-286
Starch	-98.1	V-M-MET-260-V-S-MET-260-V-M-LYS-262-V-M-GLU-263-V-S-GLU-263-V-M-PHE-264-V-S-PHE-264-V-M-ARG-269-V-S-ARG-269-V-S-VAL-273

Table 4: Interaction analysis of biosynthesized *K.galanga* compounds with IRAK4

Compound	E Value	Active site
(2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid	-74.2	E-S-ARG-347-H-S-ARG-347-V-S-GLU-225-V-S-GLN-229-V-S-GLN-232-V-S-ARG-334
6-hydroxyhexylacetate	-93.9	V-M-MET-192-V-S-MET-192-V-M-GLY-193-V-M-GLU-194-V-S-GLU-194-V-S-VAL-200-V-S-TYR-262-V-M-MET-265-V-S-MET-265-V-M-GLY-268-V-S-LEU-318
1-(5-Hydroxypentyloxy) Propan-2-One	-78.1	V-S-MET-192-V-M-GLU-194-V-S-VAL-200-V-M-GLY-268-V-M-SER-269-V-S-ASP-272-V-S-LEU-318
Benzylpenicillin	-93.4	H-M-GLU-194-H-S-LYS-213-H-M-ASP-329-V-M-MET-192-V-M-GLU-194-V-S-GLU-194-V-S-VAL-200-V-S-TYR-262-V-S-ASP-272-V-S-LEU-318
AgNP- (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid	-98.1	V-M-MET-192-V-S-MET-192-V-M-GLY-193-V-S-GLU-194-V-S-TYR-262-V-M-MET-265-V-M-GLY-268-V-S-ASP-272-V-S-LEU-318
AgNP-6-hydroxyhexylacetate	-91.5	V-S-MET-192-V-M-GLU-194-V-M-GLY-195-V-S-VAL-200-V-M-GLY-268-V-M-SER-269-V-S-SER-269-V-M-ALA-315-V-S-LEU-318
AgNP-1-(5-Hydroxypentyloxy) Propan-2-One	-86.4	V-S-MET-192-V-M-GLY-193-V-M-GLU-194-V-S-GLU-194-V-S-VAL-200-V-S-TYR-262-V-S-LEU-318
Starch	-113.1	V-S-MET-192-V-M-GLY-193-V-S-GLU-194-V-S-VAL-200-V-S-TYR-264-V-M-GLY-268-V-M-SER-269-V-S-ARG-273-V-S-LEU-318

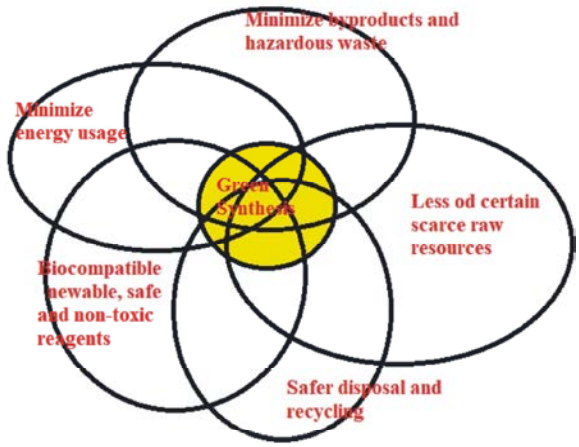

Fig. 11: Ideal sources for production of natural Nanoparticles (NPs)

kinase activity of IRAK-4 and prediction of protein/inhibitor interactions to develop potent targets having no adverse effects. Modulation of IRAK4 kinase activity presents an attractive and novel therapeutic approach for oncology and inflammatory diseases. The key pharmacophores are necessary for several classes of inhibitors for optimal protein/inhibitor interactions with nanoparticle materials of (2E, 4E, 6E)-11-Methyl Dodeca-2, 4, 6, 10-Tetraenoic Acid, 1-(5-Hydroxypentyloxy) Propan-2-One and 6-hydroxyhexylacetate are important as new anti-inflammatory therapeutic agents.

Ideal sources for production of natural Nanoparticles (NPs) are sustainable, cost-effective and renewable [36] (Figure 11). The significant recent developments related to plant-derived nanostructure (NS) provide insight into the use of plants and algae as bio- sustainable, renewable and diversified resources, in various fields including industry, medicine, agriculture and pharmaceuticals [37, 38].

The bactericidal activity on *E.coli* leading to anti-inflammation with AgNPs of Ethanolic rhizome extract from *K.galanga* has been proven in the present study. The potential activity of AgNPs from *K. galanga* confirms versatile approach of AgNPs in the bacteria exposure. The mechanism of bactericidal activity of AgNPs synthesis of Ethanolic rhizome extract from *K. galanga* may be due to the attachment of the Ag NPs to the cell wall of bacteria, disturb the permeability by penetrating into cell wall and membrane and causing intracellular ATP leakage and cell death via IRAK4 mechanism. The attachment of drug targets of AgNPs from *K. galanga* may disrupts DNA and RNA functions in *E.coli.* Previously, synthesis of metallic nanoparticles from plants like *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Centella asiatica*, Green tea leaf, *Diopyrus kaki*, *Yarrowia lipolytica*, edible mushroom and *Cladophora glomerata has* gained tremendous importance towards "green chemistry" [32, 39] Nanoparticles can easily enter cells by endocytosis and act as potential delivery vehicle for dynamic therapy [40].

CONCLUSION

The *in vitro* and *in silico* analysis of AgNPs synthesis from Ethanolic rhizome extract of *K.galanga* shows anti-inflammatory properties. Further advanced procedures has to be conducted for better understanding of control of inflammation by external sources like pollen grains, bacteria, viruses, etc.

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