Green Synthesis of Silver Nanoparticles Using Ginger Extract and Its Antioxidant (In Vitro) And Anticancer(Insilico) Study

Roja B[1], Sangavai C[2], Raja A[3], Karthik R[4]

Department of Biotechnology, DhanalakshmiSrinivasan College of Arts And Science For Women (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli (Nationally Re-Accredited with 'A' Grade by NAAC),Perambalur – 621212

ABSTRACT

Herbal drug delivery is limited by poor solubility and bioavailability which can be overcome with suitable nanomaterials that will enhance their pharmacokinetics and performance. Optimization of this green synthesis would support the production of AgNPs with great therapeutic potentials. The present work is focused on discovering a new technique for green synthesis of metal nanoparticles of ginger water and methanolic extracts. The investigated samples were used to evaluate the antioxidant capacity and antimicrobial activity. The present study reveals that to separate and detect gingerol from ginger rhizome, to synthesis, characterize silver nanoparticles (AgNPs) using gingerol, to find out antibacterial activity against *P.gingivalis,* to screen the antioxidant activities of the AgNPs, to evaluate the anti-inflammatory and anticoagulant activity of AgNpand to find out the binding efficiency of Gingerol against breast cancer target.

Keywords: Ginger rhizome, silver nanoparticles and Gingerol

INTRODUCTION

1.1. Alkaloids and pharmaceutical values

Spices and herbs have been recognized as an excellent source of antioxidant compounds such as phenols, flavonoids, tocopherols, ascorbic acid and carotenoids which have been reported to show good antioxidant activity. Plants and natural products have long played a crucial role in the treatment of various illnesses. They offer an invaluable source of compounds with a wide variety of chemical structures and biological activities and provide important prototypes for the development of novel drugs. It is difficult to overrate the importance of natural extracts as potential sources of new drugs. It is estimated that the plant kingdom comprises about 250,000 species, of which merely approximately 6% have been studied for biological activity, and about 15% phytochemically (Fabricant and Farnsworth,2001)

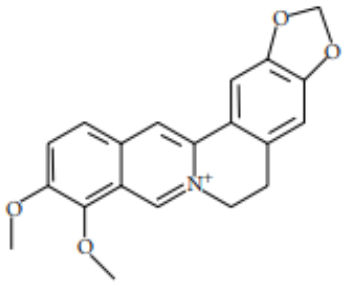
Nature has provides human a variety of useful sources mainly plants for discovery and development of drugs against dreadful diseases. Traditional herb as an effective system of treatment of cancer. Drugs from medicinal plants are found to be comparatively less toxic and side effects (Rajeshkumar et al., 2003). There are various indole alkaloids isolated from medicinal plants. From periwinkle plant Vincarosea; *Catharanthusroseus*(Apocynacea), three compounds vinblastine, vincristine and vindesine introduced a new era of the use of plant material as anti cancer agents. Vinblastin and vincristine are primarily used in combination with other cancer chemotherapeutic drugs for treatment of a variety of cancers including leukemias, lymphomas, advanced testicular cancer, breast and lung cancer and kaposi's sarcoma (Cragg and Newman, 2005) Alkaloids are a highly diverse group of compounds that contain a ring structure and a nitrogen atom. In most cases, the nitrogen atom is located inside the heterocyclic ring structure. A classification based on biosynthetic pathways is mostly used to categorize different alkaloid. Alkaloids have a wide distribution in the plant kingdom and mainly exist in higher plants, such as those belonging to Ranunculaceae, Leguminosae, Papaveraceae, Menispermaceae, and Loganiaceae. Moreover, several alkaloids exhibit significant biological activities, such as the relieving action of ephedrine for asthma, the analgesic action of morphine, and the anticancer effects of vinblastine. In fact, alkaloids are among the most important active components in natural herbs, and some of these compounds have already been successfully

developed into chemotherapeutic drugs, such as camptothecin (CPT), a famous topoisomerase I (TopI) inhibitor, and vinblastine, which interacts with tubulin (Li et al., 2007) Alkaloids are capable of modulating key signaling pathways involved in proliferation, cell cycle, and metastasis, making them the chief components of several clinical anticancer agents. Interestingly, the molecular mechanisms that these secondary metabolites act on are numerous and effective in various ways against cancer cells. For instance, paclitaxel is one of the major agents used clinically against breast cancer, ovarian cancer, non-small cell lung cancer, and prostate cancer (Millimouno et al., 2014). Berberine (Chen et al., 2007)is an isoquinoline alkaloid widely distributed in natural herbs, including RhizomaCoptidis, a widely prescribed Chinese herb. It has a broad range of bioactivities, such as antiinflammatory, antibacterial, antidiabetes, antiulcer, sedation, protection of myocardial ischemia-reperfusion injury, expansion of blood vessels, inhibition of platelet aggregation, hepatoprotective, and neuroprotective effects.

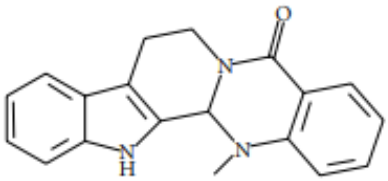
Evodiamine (Ogasawara et al., 2002), a quinolone alkaloid, is one of the major bioactive compounds isolated from the Chinese herb Evodiarutaecarpa. It possesses antianxiety, antiobese, antinociceptive, antiinflammatory, antiallergic, and anticancer effects. Besides, it has thermoregulation, protection of myocardial ischemia-reperfusion injury and vessel-relaxing activities. Evodiamine exhibits anticancer activities both in vitro and in vivo by inducing the cell cycle arrest or apoptosis, inhibiting the angiogenesis, invasion, and metastasis in a variety of cancer cell lines.

Matrine (Liu et al., 2010) is a major alkaloid found in many Sophora plants, including *Sophoraflavescens*Ait. It exhibits a wide range of pharmacological properties such as antibacterial, antiviral, antiinflammatory, antiasthmatic, antiarrhythmic, antiobesity, anticancer, diuretic, choleretic, hepatoprotective, nephroprotective, and cardioprotective effects. It has been used for treatment of bacillary dysentery, enteritis, malignant pleural effusion, and so forth in China, and the anticancer effects have also been widely studied. Although the needed concentration of matrine to inhibit cancer cell proliferation is relatively high (i.e., at millimolar level), it has no significant effects on the viability of normal cells

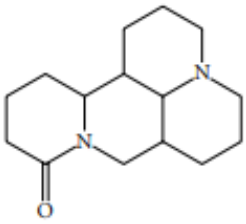
Piperine , a piperidine alkaloid isolated from Piper nigrum and Piper longum, is a compound found in famous spices that have been used for centuries. It exhibits antioxidant, antiinflammatory, antidiarrheal, anticonvulsant, antimutagenic, hypolipidemic, promoting bile secretion, and tumor inhibitory activities.Thechemopreventive effects of piperine against several kinds of carcinogen, such as benzo(a)pyrene, and 7,12-dimethyl benz(a)anthracene, show its potential as a cancer preventive agent (Selvendiran and Sakthisekaran,2004)Sanguinarine is a benzophenanthridine alkaloid isolated from the Papaveracea family. It has antibacterial, antifungal, antischistosomal, antiplatelet, and anti inflammatory properties. Sanguinarine also exhibits anticancer potentials.



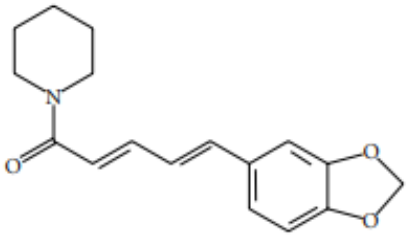
Berberine



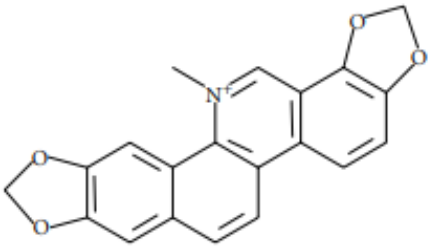
Evodiamine



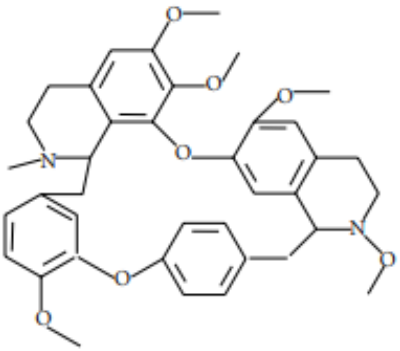
Matrine



Piperine



Sanguinarine



Tetrandrine

ALKALOIDS	**MECHANISM OF ACTION**
Liriodenine	Cleavage of caspases-3 and -9 Efflux of cytochrome C ↑Bax, ↑p53expression ↓Bcl2 and ↓surviving
Cryptolepine	↑p53 and p21 cip1\ waf1
Clausenidin	Cleavage of caspases-3 and -9 Efflux of cytochrome C ↑Bax and ↑ A paf -1
Isogravacridone chlorine	Cleavage of caspase -9
Cathachunine	Cleavage of caspeses -3, -9 and PARP Disruption of mitochondrial membrane potential Efflux of cytochrome C Activation of caspase -3 and -9 ↑ Bax and ↓Bcl -2
Brucine	↑ Bax and ↓Bcl -2 expression
Subditine	caspase -3 and -9 Efflux of cytochrome C ↑ Bax ↑ p53 expression ↓Bcl -2 and ↓Bcl –x

Scutebarbatine A (SBT-A)	Cleavage of caspeses -3, -9 Efflux of cytochrome C ↑ Bax and ↓Bcl -2
Rohitukine	Cleavage of caspeses -3, -9 Efflux of cytochrome C ↓Bcl -2
Tabernaelegantinine B Tabernaelegantine C	Cleavage of caspeses -3 and -8

1.2.Ginger extract and nanomedicine values

ZingiberofficinaleRosc. (ginger) is a rhizomatous herb belonging to the family Zingiberaceae.*Zingiberofficinale (Ginger, Zingiberaceae)* is a rhizomatous perennial plant which has been reported primarily a remedy for digestive disorders, dyspepsia, nausea, vomiting, gastritis, diarrhoea and also used for treatment of asthma, common cold disorders, nervous disease, inflammation, hepatotoxicity, diabetes, migraine, hypercholesterolaemia, helminthiasis and schistosomiasis. Ginger has been commonly used throughout the world as a spice for dietary as well as medicinal purpose since prehistoric time. Consistent with this belief, we investigated the antiproliferative and antioxidant activity.

The scientifc name of ginger is Zingiberofcinale which belongs to Zingiberaceae family containing 800 species. Ginger has an active components such as curcumin , 6-gingerol, 6-shogaol and 6-paradol. The mixture of honey and ginger has a high antimicrobial activity against Gram-positive bacteria and Gram-negative bacteria. Ginger extracts show antibacterial activity against *Staphylococcus aureus, Staphylococcus pyogenes, Staphylococcus pneumoniae and Haemophilusinfuenza*pathogens. In addition, the ginger extracts may contain compounds having therapeutic activity (Patel et al., 2011)

Ginger contains a number of pungent constituents and active ingredients. The major pungent compounds in ginger, from studies of lipophilic rhizome extracts, have yielded potentially active gingerols. The characteristic odor and flavor of ginger is caused by a mixture of gingerol and shogaol. The pungent taste of ginger is due to chemopreventive potentials of 6- gingerol present a promising future alternative to expensive and toxic therapeutic agents, non volatile phenyl propanoid-derived compounds, particularly gingerols.

The gingerol and shagelol are identified as more active agents have shown that, ginger has broad anti-bacterial activity and the ethanolic extract of ginger powder has pronounced inhibitory activities against Candida albicans(Atai et al., 2009).An important study in the favors of ginger as anti-microbial activity showed that ginger has antimicrobial activity against Ecoli, Salmonella typhi and Bacillus subtilis and ethanolic extract of ginger showed widest zone of inhibition against Salmonella typhi (Azu and On yeagba, 2007). Nanoparticles containing antimicrobial and antioxidant substances could be considered as a new trend of antimicrobial therapeutic agents for the prevention and reduction of deterioration of food and pathogenic microorganisms.Nano-sized metals have been shown to have a good antimicrobial effect and they can be introduced as a solution for the resistance problem. Moreover, nanometals have diverse applications, which can be explained by attaining a high surface area to volume ratio. Researchers mainly focus on AgNPs as they have potent antimicrobial activity (Morones-Ramirez et al., 2013)

The green synthesis of AgNPs has been developed via different biological systems, including bacteria, fungi, yeasts, algae or plants.Ginger has been found to contain a variety of bioactive compounds, including alkaloids, flavonoids, zingiberene, gingerols and shogaols, most of which exhibit antioxidant activities. Given that ginger is rich in antioxidants, the biomolecules in ginger extract are supposed to play a crucial role in the reduction of silver ions (Ag^+) to metallic AgNPs (Ag^0). Velmurugan et al. (2014) synthesized AgNPs using ginger root extract and proved their antimicrobial activity against food pathogens of *Staphylococcus* spp., Listeria spp. and *Bacillus* spp.

1.2.*Insilico*anticancer drug analysis

Modern drug discovery is primarily based on in silico studies and chemo-biological approach, where computers play an important role in the discovery of new drugs. Computers can not only save money, but also consume less time to screen the ligands on the basis of their biological structures. Use of computational

techniques in drug discovery and development process is rapidly gaining popularity, implementation and appreciation. Molecular docking has become an increasingly important tool for drug discovery for more than three decades and a great number of new drugs have been discovered and developed accordingly . Docking is mainly focused on the interaction between a small molecule and a protein at the atomic level, which allows to characterize the behavior of small molecules in the binding site of target proteins as well as to understand the inner workings of human diseases at the molecular level(Greenhough et al., 2009). Hence, docking plays an important role in the rational design of drugs.

Computational method has developed rapidly to determine the structure target in drug discovery. Structure target determination is based on ligand-protein interaction, ligand-ligand interaction, or protein-protein interaction, by docking two molecules together and studying the binding affinity between two molecules. Ligand is small molecule which is usually known as structure target, docking gives the advantage as virtual screening to sort out the molecules which are predicted having potential activity; virtual screening of compound libraries has become a standard technology in modern drug discovery pipelines (Seeliger and de Groot, 2010). Virtual screening can reduce the hardship and less expensive in determination of structure target. Due to this reason, in this experiment, vicanicine from *R. javanica*which has been isolated was analyzed by *in silico*through docking approach.

Cancer is the leading cause of morbidity and mortality worldwide.The body is made up of many types of cells can grow and divide in a controlled way to produce more cells as they are required to keep the body healthy. When cell become old or damaged, they die and are replaced with new cells. However, sometimes this orderly process goes wrong. The genetic material of a cell can become damaged, producing mutations that affect normal cell growth and division. Generation of free radicals or reactive oxygen species (ROS) during even normal metabolism can cause extensive damage to cells and tissues.

Cancer is an uncontrolled growth of cells resulting in lack of differentiation and ability to invade local tissues and metastasis which are reproduce individually throughout the body. During metastasis, cancer cells enter the blood stream and are carried to distant parts of the body where they form other similar growths. Cancer may affect people at all ages, even fetuses, but the risk of most varieties increase with age (Roger Walker, 2003).

Many studies reveal the apoptotic interaction between p53 and Bcl-2 gene expression in several types of human and experimental cancerous models. p53 is one of the most promising cancer therapeutic targets and serves as a gatekeeper of the cell, protecting it from genetic instability and acting to sense multiple stress signals, some of which include DNA damage, activation of oncogenes and hypoxia(Hong et al.,2014) . On the contrary, Bcl-2 family proteins are key regulators of apoptosis, which include both anti- (Bcl-2, Bcl-xL) and pro-apoptotic proteins (Bax, Bak, Bid, Bim and Bad) and over expression of these anti-apoptotic proteins leads to cancers by preventing apoptosis. It is considered as an attractive target to develop a new generation of therapeutics for the treatment of cancer

*In silico*computer-based modeling technologies have also been applied in Simulation of oncological clinical trials exploiting grid computing infrastructures, such as the European Grid Infrastructure, for improving the performance and effectiveness of the simulations. (Athanaileas and Theodor,2011).*In silico*study in medicine is thought to have the potential to speed the rate of discovery while reducing the need for expensive lab work and clinical trials. One way to achieve this is by producing and screening drug candidates more effectively. the protein docking algorithm EADock (see Protein-ligand docking), researchers found potential inhibitors to an enzyme associated with cancer activity *in silico*. Fifty percent of the molecules were later shown to be active inhibitors *in vitro*(Röhrig Ute et al., 2010).

MATERIALS AND METHODS

3.1.Extraction procedure-Ultrasonication

Roots were collected from local market, Tiruchirappalli and washed well, disinfected with 70% ethanol. The skin removed ginger finely chopped and dried. The roots were added with methanol and water and left for 3 days under refrigeration. The solvent samples (99% methanol and water) were first prepared in 100ml

conical flasks and covered by rubber corks to prevent their vaporization at room temperature 5gm of crushed rhizome was weighed for every solvent sample and added to the solvents in the conical flasks. Phyto constituents was extracted using solvents by subjecting the mixture of powder and solvent in conical flasks to sonication in the bath sonicator for 20 minutes at a frequency of 100 Hz. The samples were then filtered using Whatman filter paper 4.

3.2.Batch distillation

Solvent recovery is done using a lab scale. Sample is taken in a 100ml round bottom distillation flask and heated at the solvent boiling point until the solvent is collected by condensation in the receiver. The condensate is then collected and the recovered solvent is eluted with columchromatography measured by a measuring cylinder. The quantity of extract left in the pot is separately measured. Solvent recovered is calculated by the following formula

% Solvent recovery = Amount of condensate recovered / Amount of solvent taken initially for extraction * 100

3.3.Detection of Alkaloids

Extracts were dissolved individually in dilute hydrochloric acid and filtered. The filtrates were used to test the presence of alkaloids.

3.3.1.Dragendorff test

Filtrates were treated with **Dragendorff**reagent. Formation of a yellow cream or brown precipitate indicates the presence of alkaloids.

3.3.2.*TLC analysis*

Extract of ginger's rhizomes is chro-matographed on a pre-coated TLC silica gel plate (silica gel G 06o F_{254} plates). The TLC plates were put inside the development tank for the solvent to run up. N-hexane:diethyl ether (40:60 v/v) was used as developing solvent system. Then the plates were taken out before the solvent front reach to the top. The dried plates were then visualized by heating at 110 ℃ after spraying the reagent. The reagent used was sulfuric acid and vanillin.

3.3.3.Steroids test

2 ml of acetic anhydride was added to 0.5 g of ginger extract and 2 ml of sulphuric acid was added by the sides of the test tube a color change was observed to violet or blue-green which showed the presence of steroids

3.3.4.Phenolic compound

To 2 ml of extract, 2 ml of 5% aqueous ferric chloride were added; formation of blue colour indicated the presence of phenols in the sample extract

3.4.Standardization of silver reduction

Gingerol 1% was taken and mixed with 5mM Silver nitrate at different ratio in final volume of 10 mL. The ratio of ginger extract and $AgNo_3$ were as follows 1:9, 2:8, 3:7, 4:6 and 5:5. The mixture was incubated under room temperature and changes of color versus time was noted.

3.5.Synthesis of AgNPs

The 1% gingerol separated from TLC plate was mixed with 5mM Silver nitrate solution in water in the ratio of 1:9 and was kept in the dark for 24h in room temperature in order to produce and settle silver nanoparticles.

3.6.UV-Visible Spectroscopy Characterization

The formation of AgNPs was confirmed using UV-Visible spectrophotometer (CECIL CE 2041 2000 SERIES). Exactly, 5 mL of the diluted supernatant of the WMRE-AgNPs sample was placed in a quartz cuvette with a 1 cm path length and inserted in a UV-Vis spectrophotometer in the wavelength range of 300–700 nm to obtain the UV-Visible spectra of the sample.

3.7.Scanning Electron Microscopy (SEM)

The sample was prepared by centrifuging the cell filtrate initially at 5000 rpm for 20 min and then the supernatant was collected and centrifuged at 12,000 rpm for 15min. the pellet was washed with ethanol and air dried and making a smear with the pellet obtained. This was then subjected to SEM analysis. sample is

examined in field emission scanning electron microscope (Model: SIGMA VP, make: Carl Zeiss Microscopy GmbH, Germany; operated at 15 kV) equipped with X-ray energy- dispersive spectrometry system.

3.8.Antibacterial activity

Bacterial culture

The bacterial culture of were purchased from ATCC and maintained on Blood agar plates.

Bioassay of gingerol

TLC plates in a narrow band and eluted using the chloroform-acetone ethylacetate (4:1:2) mobile solvent systems. The developed plates were concentrated several times using N-hexane:diethyl ether (40:60 v/v) mobile phase and dried under room temperature in a chamber. TLC fractions collected by *Rf*values and redissolved in ml ethanol. 24 h old cultures of *P.gingivalis,* swabbed on agar plate to obtain lawn culture. Thereafter, the sterile disc loaded with 100 µL of TLC fraction and were kept on MH agar plates and incubated overnight at 35°C under anaerobic condition. The active fraction eluted from TLC plate determined by zone of inhibition.

3.9.Ferric-Reducing Antioxidant Power Assay (FRAP)

The Ferric-Reducing Antioxidant Power Assay (FRAP) was determined according to a method of Zhang, et al. (2018) The entire experiment was carried out at room temperature and in a dark environment. After the reaction of 100 µL of sample solution with 3 mL of FRAP reagent incubated at 37°C for 30 minutes and the absorbance value was determined at 593 nm. A standard curve was prepared using different concentrations (0.05-0.4 mM $Fe^{2+}\cdot L^{-1}$) of $FeSO_4\cdot 7H_2O$.

3.10.anti-inflammatory effects

To evaluate the anti-inflammatory effects of the extracts, the protocol described by Padmanabhan and Jangle was used. A volume of 100µL of extracts (aqueous), Nanoparticle and diclofenac sodium at concentrations (100 μg/ml) was mixed with 1 ml of aqueous solution of bovine serum albumin (5%) and incubated at 27°C for 15 minutes. The mixture of distilled water and BSA constituted the control tube. Denaturation of the proteins was caused by placing the mixture in a water bath for 10 minutes at 70°C. The mixture was cooling inside the ambient room temperature, and the activity each mixture was measured at 660 nm. Each test was done three times. The following formula was used to calculate the activity

inhibition percentage= C-T/C x 100

3.11.Blood clotting time measurement

In vitro Clotting time measurement was carried out using a modified method s reported by Osoniyi and Onajobi. Clotting tubes containing 0.1ml each of crude extract and ZnO nanoparticle suspended in Phosphate Buffered Saline (PBS) and Acid citrate dextrose (anticoagulant) were incubated in a water bath at 37 °C. Freshly drawn blood (0.5ml) was carefully transferred by running it down the side of the tube into the contents of each of the incubated tubes, while simultaneously starting a stopwatch. At 30s interval, the tubes were gently slanted to an angle of 45° to check for blood clot formation. The time for the first observation of clot was recorded, and the slanting at interval continued until the tubes could be inverted without blood flowing. The stopwatch was stopped instantly, and the time was recorded as the final clotting time

3.12. *In Silico*analysis

3.12. 1.Selection and preparation of the ligands

For the current study, gingerol is selected. The 2D structures of the selected phytochemicals were represented in FIGURE 1. The corresponding 2D structures of the eight phytochemicals were sketched on ChemSketch

3.12.2. Target preparation: The X-ray crystal structure of human breast cancer protein EGFR (PDB ID: 1M17) complexed with Erlotinib, was retrieved from the protein data bank. Delete co-crystal ligand and unwanted water molecules were removed from crystal structure of EGFR

3.12.3. Molecular docking

Molecular docking is one of the important methods for methods for identification of protein-ligands interaction. In this studies SWISS DOCK were used to perform docking studies. Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. Finally we select best ligand based on glide score and glide energy.

RESULTS AND DISCUSSION

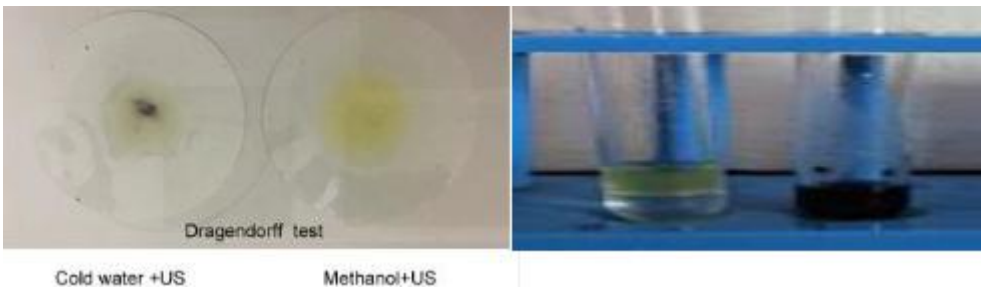
Ginger rhizome (plate 1) processed and extraction was taken by ultrasonication and purified by column chromatography. The yield of extraction followed by distillation was 40 and 65% respectively for methanol and water (table 1). Phytochemical screening of methanolic extract showed presence of alkaloid, phenol and steroids where as phenol is negative in water extract (plate 2a-b). Formation of creamy yellow and brown precipitate by the addition of iodine indicate presence of different alkaloid among methanol and water. Gingerols a phenolic compound was detected in both extract .formation of light violet spots (plate 2c) which was equal to standard spot at *Rf*0.36 . Indeed, results showed no more spots in regenerated in column purified methanol extract and two fractions among water extract were detected. Based on standard gingerol spots were identified. Ginger is abundant in active constituents, such as phenolic and terpene compounds. The phenolic compounds in ginger are mainly gingerols, shogaols, and paradols (Prasad and Tyagi, 2015).

Table 1. Extract quantification and phytochemical test

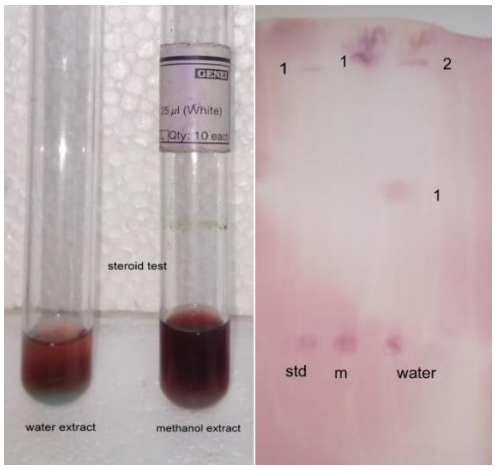
Sample	Alkaloid	Steroid	Phenol	Volume of condensate	Vol of extract taken	Yield %
Methanol	+	+	+	40	100	40
Water	+	+	-	65	100	65

Plate 1. Processing and extraction of ginger rhizome

Plate 2. Alkaloid detection test



a)Dragendorff test b)Phenol test



b) Steroid test c) gingerol detection by TLC

4.2.synthesis of AgNp and Antibacterial activity

Silver nanoparticles (b-AgNPs) have been synthesized using Ginger rhizome compound gingerol , which acted as reducing as well as stabilizing/capping agent. The one step synthesis reaction was carried out at ambient conditions (room temperature and atmospheric pressure) in water solvent, a universally accepted solvent. Appearance of ash yellow to brown color in a time dependent manner was observed in 1:9 ratio with in 30 minute incubation (Plate 3a) indicates the formation of silver nanoparticles, which was further confirmed, by the appearance of absorption peak around 465 nm, measured by UV visible spectroscopy (Figure 1). The highest absorbance peaks (at 465 nm) at 24 h were observed for reduced $AgNo_3$, indicating the formation of AgNPs. in green synthesis of metal nanoparticles, natural material extract acts as a reducing agent for the generation of metal nanoparticles (Morones et al. 2005). The concentration of the reducing materials might play a crucial role in the determination of shape, size and reaction time. A broad peak around 465nm is a characteristic band for the Ag, arising from the excitation of longitudinal plasmon vibrations of AgNP was recorded also reported by Ukiya*et al.,* (2001). The SEM image(plate 3b) analysis reveals that the particles are monodisperse spherical and 60 nm in diameter.

The antimicrobial activity of ginger extract and silver nanopartile against tooth decaying *P.gingivalis*(plate 3c) determined by disc diffusion method. The findings of the present study revealed that *Zingiberofficinale*contain potent antimicrobial property against anaerobic tooth infecting *P.gingivalis.* The antimicrobial activity of the ginger extracts (atalyz extract) was initially evaluated by disc diffusion method (plate 3d). The zone of inhibition was 16 mm against extract and 20 mm against Green nanoparticle. Clorohexidin positive control gave 10 mm inhibition (table 2). Further the bioassay of TLC fraction reveals antibacterial activity against ***P.gingivalis***and it was more active than positive control cyclohexidin mouth wash. Most of the activities were tested against aerobic clinical pathogens. But first time in this study the activity of Gingerols was tested against anaerobic hemolytic pathogen.

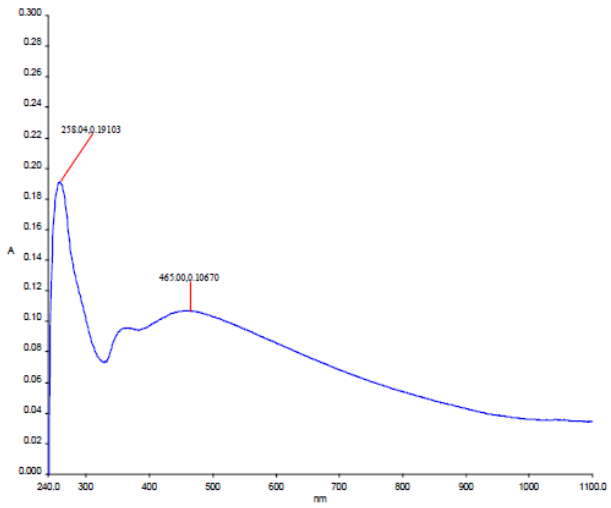
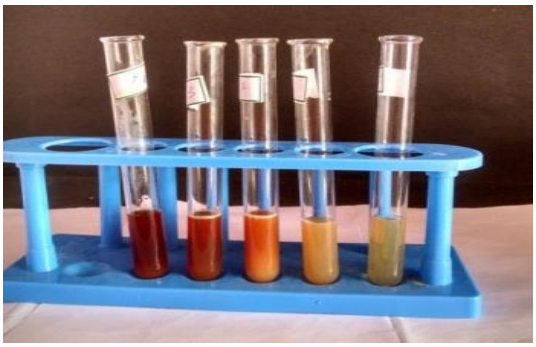


Figure 1. UV spectrum of reduced silver nitrate

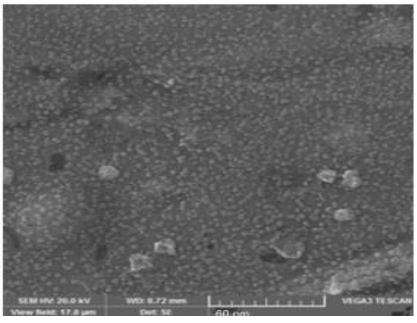
Table 2. Antibacterial activity of gingerol and AgNP

Organism	AgNP	Gingerol	NC AgNo3	NC methanol	PC
P.gingivalis	20mm	18mm	≤5mm	-	10mm

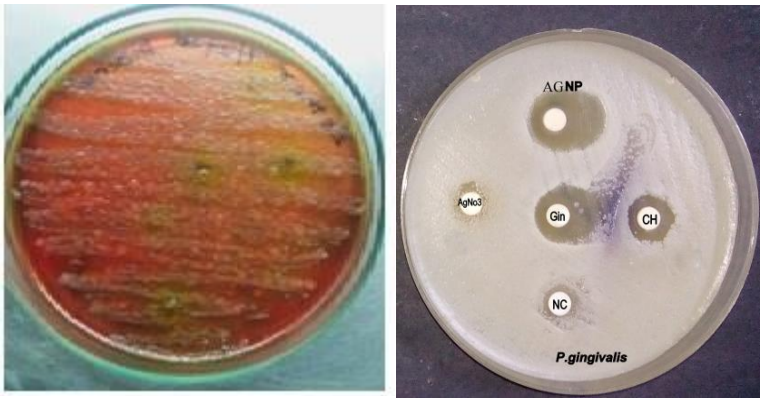
Plate 3.Antibacterial activity of TLC fraction and silver nanoparticle



a)standardization of AgNp reduction



b)SEM image of synthesized AgNP



c)Hemolytic*P.gingivalis* d)Antibacterial activity

4.3.antioxidant, anti inflammatory and anticoagulant activity

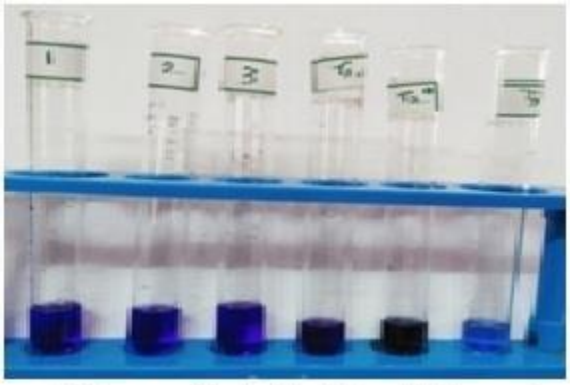
Figure 2 result os FRAP showed antioxidentavtivity of Gingerol mediated Np have higher radicle scavanging activity than standard. Extracted ginger and silver ions were given significant antioxidant property (Plate 4).The 10 µg of AgNp and Gingerol is found to be equivalent to 100 µg ascorbic acid. All of the extracts showed positive results in FRAP assay, The antioxident activity estimated as 120, 260, 320 µM among standard and 336 and 280µM among AgNp and ginger extract . FRAP assay is based on the ability of the antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe2+-TPTZ complex with an absorption maximum at 593 nm. Previous work where positive strong relationship of anthocyanin extract with FRAP assay were reported (Yang and Zhai, 2010). It was reported by Li et al during 2016 that the dried ginger have decreased antioxidant activity, because the processing could change gingerols into shogaols.

Table 3 shows the anti-inflammatory property was tested by gingerol and silver nanoparticle by using BSA (plate4). The synthesized nanoparticles show the quality of anti-inflammatory activity in the range of 61%, respectively. The extract shows a similar percentage of inhibition when compared to the standard solution diclofenac (58%) the most commonly used drugs for the inflammation. Silver nano particles produced from Gingerol showed the anti-inflammatory activity and thus can be considered as potent anti-inflammatory agent. Luettig et al reported that ginger and its active constituents possessed anti-inflammatory activity associated with the inhibition of Akt and NF-κB by phenol and terpenoids of ginger.Similarly Zhang et al (2018) found oral administration of NPs-PEG-FA/6-shogaol encapsulated in a hydrogel system significantly alleviated colitis symptoms by regulating anti-inflammatory factors

Anticoagulant activity of green synthesized nanoparticles was tested by addition of nanoparticles to human blood samples and further observation(plate 5). The control in vial A began to coagulate within 10 min of incubation. The blood sample thickened over time and finally formed thick blood clot after 30 min. On the other hand, the blood sample with addition of nanoparticles showed no significant changes and eventually no mark of coagulation was observed after 60min of incubation. The activity was retained for 24h at room temperature. The synthesized silver nanoparticle is acted as good anticoagulant equivalent to EDTA (figure 3). It was reported that the nano-particles inhibits the conversion of

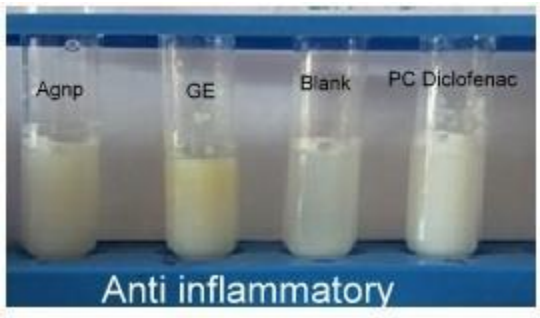
prothrombin into thrombin which is the key factor for producing insoluble strands of fibrin and catalyzing other coagulating factors (Lateef et al., 2016).

Plate 4. Antioxidant activity of AgNp and Gingerol



FRAP assay 1-3 standard; T1 np T2 gingerol

Plate 5 Anti inflammatory and anti coagulant assay



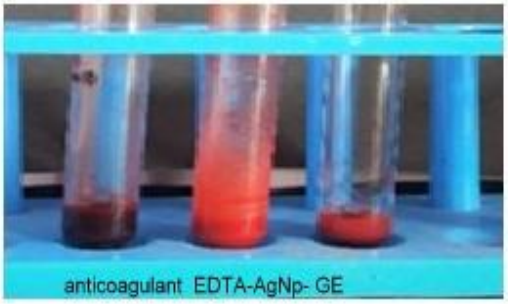


Table 3. Anti- inflammatory test for ZnO NP

S.NO	**SAMPLES**	**%**
1.	Diclofenac	58
2.	Test AgNP	61
3.	Gingerol	58

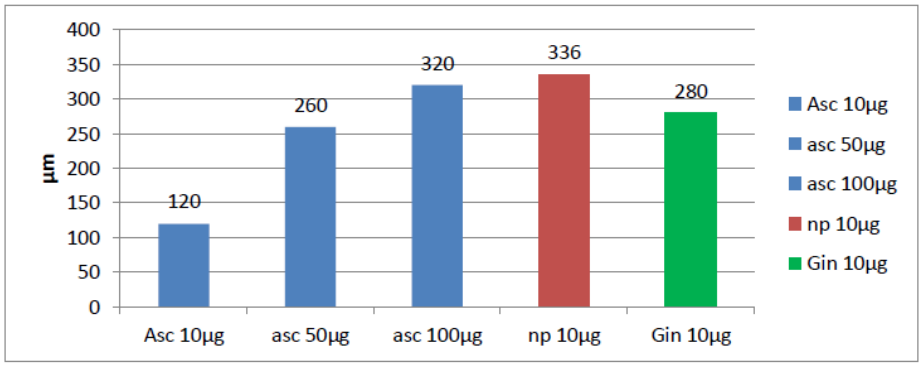


Figure 2. antioxidant activity of ginger rhizome and NP by FRAP method

4.4. Molecular docking of Gingerol with EGF

The active sites were predicted from the target ID's EGFR and Active sites were selected for targets. were performed with 6-gingerol and results were analyzed based on C-Docker energy, CDocker interaction energy, Binding energy, H-Bond, Ligand energy, protein energy and complex energy. Binding energy and H-Bonds in the active site indicate the effective stable conformation of the 6-gingerol (figure 3). The 6-gingerol shown maximum of -21 score with three hydrogen bond. The hydrogen bond was predicted among THR 830-O8 4.6A, ASP831-O11 4.7A and PHE 699-O8 5.4A. Followed by cluster V5 shows scoring of -13.7664 hydrogen bonded with ASP831, GLU 738, GLU780 and V10 Scoring -18.3105 hydrogen bonded with ASP831, ASP746, LEU768(table 3).

FIGURE 3. Scoring of Docking EGFR with 6-gingerol

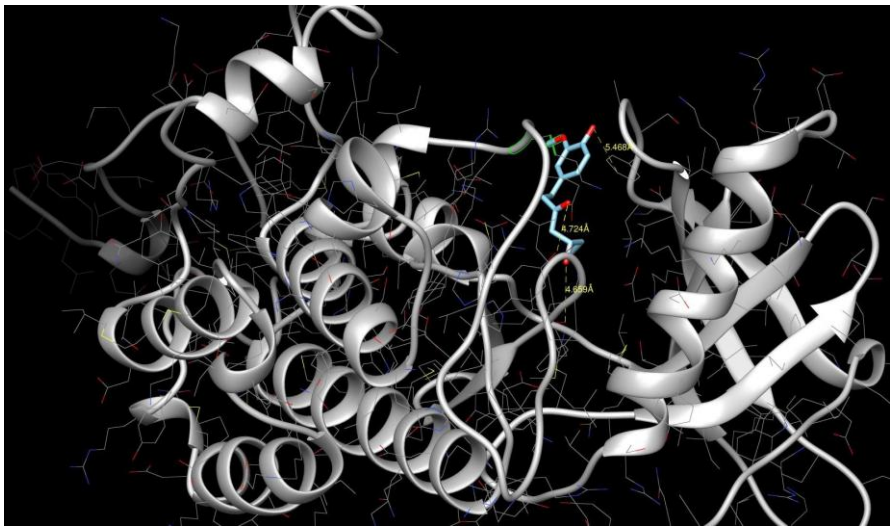
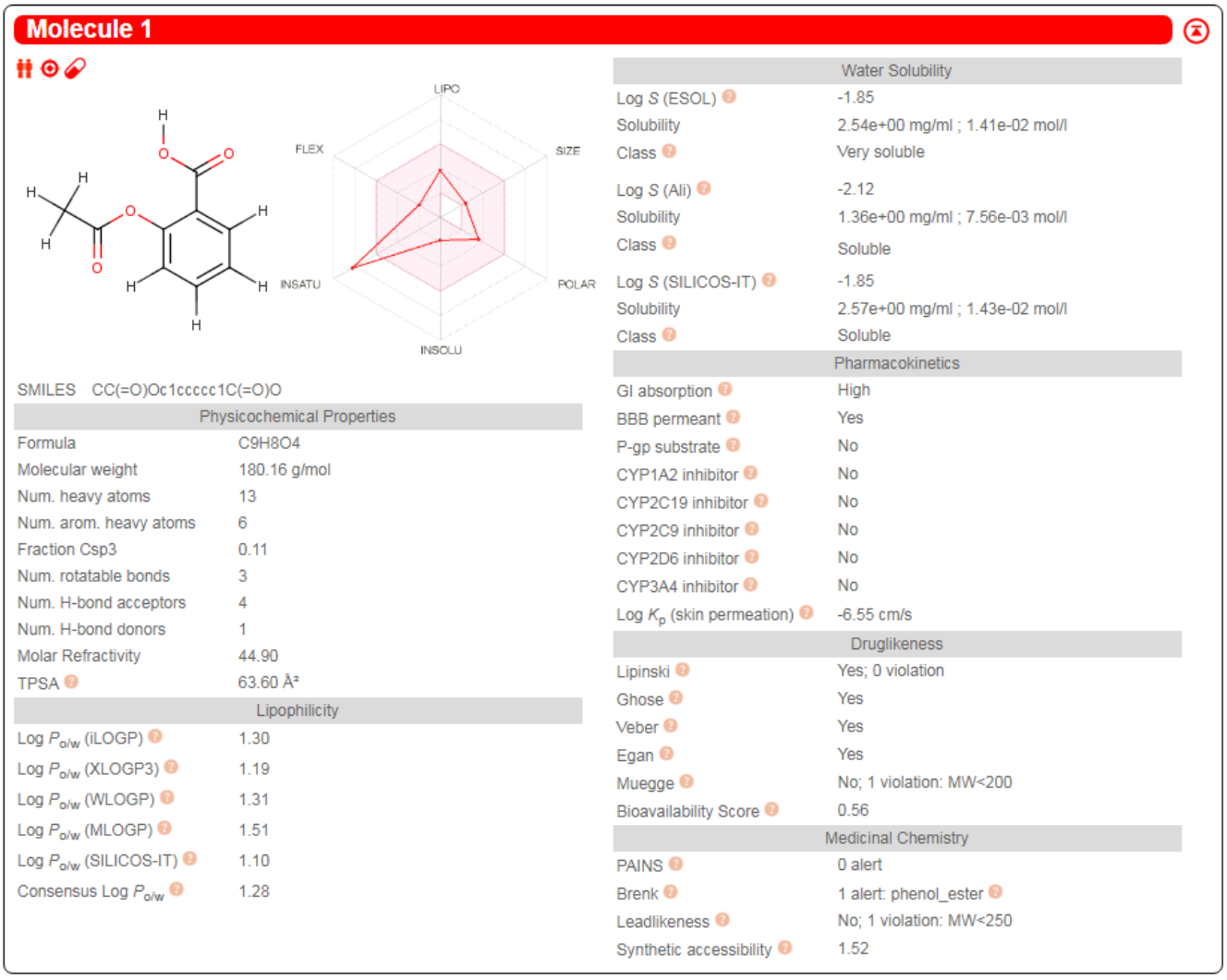


Table 3. Docking score and hydrogen bonding of Ligand with EGFR

Cluster	Docking energy	Number of H-Bond	H-Bond interaction	Full fitness
V1	-21	3	THR 830-O8 4.6A ASP831-O11 4.7A PHE 699-O8 5.4A	-2238.7441--
V5	-13.7664	3	ASP831-O18 3.6A GLU 738-O20 5.3A GLU780-O11 12.2A	-2236.04
V10	-18.3105	3	ASP831-O8 2.8A ASP746-O11 16.3A LEU768-O20 5.5A	-2236.2285



SUMMARY AND CONCLUSION

In the present study, green synthesis of silver nanoparticles with a particle size of 60 nm were synthesized using Zingiberofficinale root extract as a reducing and capping agent.the extract collected by ultrasonication and phenol, alkaloid and steroid were detected. Methanloic extract and water extract showed presence of gingerol detected and compared with known standard. The active ingredient Gingerol was collected and used for synthesis of silver nanoparticle. The AgNp and gingerol were further tested for antioxidant, antibacterial, anti-inflammatory and anticoagulant. Both gingerol and AgNp showed a potent antioxidant, antibacterial, anticoagulant and moderate anti-inflammatory property. Further ADME results shows that the compound have 4 hydrogen acceptor, hydrogen donar and 3 rotatable bonds. docking of gingerol with EGFR showed ad the strongest binding energy with formation of hydrogen bond with . Therefore this study concludes delivry of gingerol with silver nanoparticle might be an ideal source for cancer treatment.

REFERENCES

Azu N, On yeagba R. (2007) Antimicrobial Properties Of Extracts Of Allium cepa (Onions) and Zingiberoficinale (Ginger) On *Escherichia coli, Salmonella typhi and Bacillus subtilis*. The Internet Journal ofTropical Medicine; 3:1-10. Atai Z, Atapour M, MohseniM.Inhibitory effect of Ginger Extract on *Candida Albicans*. J Applied Sci 2009; 6: 1067-1069

Bellik Y (2014)Total antioxidant activity and antimicrobial potency of the essential oil and oleoresin of Zingiberofficinale Roscoe. Asian Pac J Trop Dis. 4(1): 40–44.

Chen J, Zhao H, Wang X, Lee F. S. C, YangH, and ZhengL."Analysis of major alkaloids in Rhizomacoptidis by capillary electrophoresis-electrospray-time of flight mass spectrometry with different background electrolytes," Electrophoresis, 2008, 29(10): 2135–2147.

Fabricant DS, Farnsworth NR The value of plants used in traditional medicine for drug discovery. Environ Health Perspect 2001, *109*(1): 69-75.

GargiDinda, DipankarHalder, AtanuMitra, Nabanita Pal & D. K. Chattoraj (2019) Phytosynthesis of silver nanoparticles using *Zingiberofficinale*extract: evaluation of their catalytic and antibacterial activities, Journal of Dispersion Science and Technology, DOI: 10.1080/01932691.2019.1653194

Greenhough A, Smartt HJ, Moore AE, et al. A COX-2/PGE 2 pathway: key roles in the hallmarks of cancer and adaptation to the tumour microenvironment. Carcinogenesis 2009; 30: 377-86.

Li W., Shao Y., Hu L. (2007) "BM6, a new semi-synthetic Vinca alkaloid, exhibits its potent *in vivo* anti-tumor activities via its high binding affinity for tubulin and improved pharmacokinetic profiles," *Cancer Biology and Therapy*, 6(5):787–794,

Li Y., Hong Y., Han Y., Wang Y., Xia L. Chemical characterization and antioxidant activities comparison in fresh, dried, stir-frying and carbonized ginger. J. Chromatogr. B. 2016;1011:223–232.

Liu T. Song Y, Chen H, Pan S and X. Sun. “Matrine inhibits proliferation and induces apoptosis of pancreatic cancer cells in vitro and in vivo,” Biological and Pharmaceutical Bulletin,2010, 33(10): 1740–1745.

Mariadoss AV, Kathiresan S, Muthusamy R, Kathiresan S. (2013) Protective effects of [6]-paradol on histological lesions and immunohistochemical gene expression in DMBA induced hamster buccal pouch carcinogenesis. Asian Pac J Cancer Prev.14(5):3123-9.

Millimouno F.M, Dong J, Yang L, Li J, Li X. Targeting apoptosis pathways in cancer and perspectives with natural compounds from mother nature. Cancer Prev. Res. 2014, 7: 1081–1107

Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramı´rez JT, Yacaman MJ (2005) The bactericidal effect of silver nanoparticles. Nanotechnology 20:2346–23453

Morones-Ramirez J.R, Winkler J.A, Spina, C.S, Collins J.J. Silver Enhances Antibiotic Activity Against Gram-negative Bacteria. *Sci. Transl. Med.* **2013**, *5*:190ra81

Mustafa I, Nyuk Ling C, Sharida F, Arulselvan P(2019) Comparison of Phytochemicals, Antioxidant and Anti-Inflammatory Properties of Sun-, Oven- and Freeze-Dried Ginger Extracts.Foods. 8(10): 456.

OgasawaraM, Matsunaga T, Takahashi S, Saiki I and Suzuki H. “Anti-invasive and metastatic activities of evodiamine,” Biological and Pharmaceutical Bulletin, 2002, 25(11): 1491–1493,

Patel, R.V., Thaker, V.T., Patel, V.K.: Antimicrobial activity of ginger and honey on isolates of extracted carious teeth during orthodontic treatment. Asian Pac. J. Trop. Biomed.2011, 1: 58–61.

Riaz H, Almas Begum, Syed AtifRaza, Zia Mohy-Ud-Din Khan, HamadYousaf and Ayesha Tariq Antimicrobial property and phytochemical study of ginger found in local area of Punjab, Pakistan. *International Current Pharmaceutical Journal*, 4(7): 405-409.

Selvendiran K. and D. Sakthisekaran, “Chemopreventive effect of piperine on modulating lipid peroxidation and membrane bound enzymes in benzo(a)pyrene induced lung carcinogenesis,” Biomedicine and Pharmacotherapy, 2004, 58(4):264–267

Velmurugan P, Anbalagan K, Manosathyadevan M, Lee KJ, Cho M, Lee SM, Park JH, Oh SG, Bang KS, Oh BT. Green synthesis of silver and gold nanoparticles using Zingiberofficinale root extract and antibacterial activity of silver nanoparticles against food pathogens. Bioprocess Biosyst Eng. 2014 ;37(10):1935-43. Velmurugan P, Anbalagan K, Manosathyadevan M. Green synthesis of silver and gold nanoparticles using Zingiberofficinale root extract and antibacterial activity of silver nanoparticles against food pathogens. Bioprocess and Biosystems Engineering, 2014; 37(10):1935–1943

Zhang, M, Xu C, Liu D, Han M.K, Wang L, Merlin D. (2018)Oral delivery of nanoparticles loaded with ginger active compound, 6-shogaol, attenuates ulcerative colitis and promotes wound healing in a murine model of ulcerative colitis. J. Crohns Colitis. 12, 217–229.