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# Anti-gout arthritic activities of Ethanolic and Zinc oxide Nanoparticle extracts of *Citrullus colocynthis*- An *In vitro* and *Insilico studies*

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## Abstract

The aim of the present investigation is to evaluate and compare the anti gout activity of the ethanolic stem extract of *Citrulluscolocynthis* (ESECC) with that zinc oxide nano particle loaded ethanolic extract of *Citrulluscolocynthis*(ESECC ZnNP).The antioxidant activity in terms of DPPH and Hyrogen peroxide scavenging and The *invitro*anti-gout arthritic activity in terms of their inhibitory potential of xanthine oxidase, protein denaturation and membrane stabilization were evaluated for these extracts using standard protocols. *In silico*anti-gout arthritic activity was examined by molecular docking using the 16 phyto ligands derived from GC Ms analysis with that of the TLR2 and TLR4 receptor proteins, in order to find out the antagonistic nature in arresting the immunological signaling process. Among these extracts the Zinc oxide loaded nano particle of *Citrulluscolocynthis*exhibited better anti gout activity in terms of inhibiting the xanthine oxidase, protein denaturation and exhibiting higher antioxidant and membrane stabilizing potential.In the docking analysis based on the docking score and amino acid interactions three ligands namely methylvinyphenol, pentadecanoic acid and phytol has the potential to inhibit the TLR2 and TLR4 receptors there by serves as a better antagonistic ligand to suppress immunological reactions observed in the gouty arthritis. Itmaybe concluded that the Zinc oxide loaded nano particle of *Citrulluscolocynthis can be* further developed into a effective drug for the management of gouty arthritis due to its antioxidant and suppressive nature of immunological reactions.

**KEY WORDS: A**nti-gout arthritic, , Membrane stabilization TLR 2,TLR4 and Xanthine oxidase

## Introduction:

Nanoparticles can be synthesized from various base materials which include chemical, biological substances. The physical and chemical methods which are being usedare reduction by various solutions, radiation assisted preparation,thermal decomposition, microwave heating and electrochemical sonication and so on. At present biological methods are now widely being utilized since the nanomaterials prepared by chemicalprocedure utilizes highly toxic chemicals which causes environmental toxicity.The new approach of “green

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nanotechnology" utilizes the organic method of nanoparticle synthesiswhich is accomplished without the use of toxic chemicals. The plant The plant or microbial moderate methods are widely being keep to as they are simple, low-cost, sustainable and permitquick results. As, plants are rich in various proteins and secondary metabolites including quinones, flavonoids, saponins, and terpenoids, which are involved in the equalization or reduction of the nanoparticles(Rajesh Kumar et al.,2019).

Zinc Oxide nanoparticles play a vital role in diagnostics, biomolecular detection and microelectronics.Several conventional methods are used for synthesis of zinc oxide nanoparticles like chemical reduction, laser ablation, solvothermal, inert gas condensation sol-gel method (Imran Khan et al., ).The process of inflammation is a complex response and is produced by the vascular tissue to protect the tissues against the harmful pathogens, endogenous substances and irritants which causes redness, swelling and pain.However, if prolonged and untreated, it may cause chronic inflammatory condition due to inflammation as seen in several auto immune disorders such as arthritis and asthma (Meyer et al.,2005).The arthritis refers to the inflammation of joints, and irrespective of its etiologic, like infection, genetic defect or some other cause which leads to disability due to the destruction of bone and cartilage.Gouty arthritis is a metabolic disorder, caused by obesity, purine rich diet, high blood pressure, and alcohol consumption which leads to increased xanthine oxidase expression and hyperuricemia(Koeberle& Werz,2014). The increase in concentration of uric acid in blood promotes the formation of monosodium urate crystals (MSU) which gets deposited in the articular cavity which further initiates the acute inflammatory reactions leading to the degenerativecondition called gouty arthritis. The acute inflammatory reactions are multifaceted which include swelling, pain neutrophils infiltration which is followed by the release of cytokines (IL-1$\beta$, TNF-$\alpha$),chemokines, prostaglandins, free radicals and matrix metalloproteinase which ultimately destroys the tissues and bones by triggering the inflammatory response(Walizaansar&shyamsreeghosh, 2016).

*Citrullus Colocynthis* L. species (Cucurbitaceae) which are used in traditional medicine to cure numerous diseases are regularly distributed in the Mediterranean region, and in Tunisian arid regions, particularly in Gaffs. In recent years, *Citrulluscolocynthis*known as "Hadaj" in Tunisia, are used as anti-inflammatory, antioxidant, antidiabetic, antibacterial, anticancer, and analgesic agents (MoncefChouaibi , et al.,2020). *Citrullus colocynthis* is a strong free radical scavenger and act as antioxidant. To this reaction it can manage free radical damage and treat oxidative stress-related diseases. Its antioxidant probable is important as ROS give to inflammation, cancer, tissue injury and numerous illnesses. Various parts C. *colocynthis* such as stem, roots, fruit and leaves has exhibit anticancer potential against many cancers in countless studies. Its alkaloid rich fruit extract has shown hopeful anticancer activity in breast and liver cancer cells. (Nakamura2012).With the above scenario we made an attempt to evaluate the Anti-gout arthritic activities of Ethanolic and Zinc oxide Nanoparticle extracts of *Citrulluscolocynthis.*

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## Materials and Methods:

### Chemicals

The protein Bovine serum albumin and enzymes such as Xanthine oxidase and trypsin were purchased from Hi media chemicals. The chemicals such as ethanol, methanol, acetyl salicylic acid allopurinol, and other analytical chemicals were obtained from SD Fine Chemicals Ltd.

### Preparation of Ethanolic extract of *Citrullus colocynthis*stem (ESECC)

*Citrullus colocynthis*stem were collected and dried. After the collected stem were washed 2-3times using distilled water then dried it in shade for 7–14days.The ethanolic extracts of *Citrullus Colocynthis* stemwere prepared by soaking in 90% (v/v) ethanol separately for three days in room temperature and following cold maceration process. The extracts were filtered and the excess solvent present in these extracts were removed by vacuum evaporation method and the dry extracts yield was calculated. The yield of ethanolic extract of *Citrullus Colocynthis* (EECC) stem is found to be 1.34%(w/w).

### Synthesis of *Citrulluscolocynthis*stem loaded zinc oxide nanoparticles (ESECC ZnNP)

The shade dried fruits were fine powdered, then 1mg of *c. colocynthis* stem weighted and boiled with 100ml distilled water then the extract was filtered by using Whatman filter paper. About 0.594g of Zinc nitrate was prepared using 65ml of distilled water and 35 ml of the*citrulluscolocynthis*stem extract was added to this solution and was placed in the orbital shaker. Colour change of the solution was noted every 2 hr. Readings were recorded every 2 hr in U V Spectrophotometer and after around 36 hours, centrifugation was done at 7000 rpm for 10 minutes. Zinc Oxide nanoparticles pellets reinforced with *C.colocynthis*stem were obtained after centrifugation.

### Characterization of *Citrullus colocynthis* stem loaded zinc oxide nanoparticles (ESECC ZnNP)

The synthesis of NPs solution initial characterized using UV-visible spectroscopy. About 3 ml of the solution is taken in cuvette and scanned in double-beam UV-visible spectrophotometer from 200 nm to 700 nm wavelength. The results were recorded for the graphical analysis.

### Antioxidant activity

### DPPH scavenging activity

DPPH scavenging activity ofESECC and biosynthesized ZnNPwas measured using DPPH radical scavenging assay by (Keshari et al., 2020) with slight modification. Briefly, 0.1 mM DPPH solution in methanol was prepared and different concentrations of ESECC, ZnNP and ascorbic acid (e.g., 100,200.400,800 and 1000µg/mL) were mixed with DPPH solution to attain the respective final concentration. Ascorbic acid considered as standard and DPPH solution without sample was used as a blank. The mixture solution was vortex-mixed and

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then incubated for 30 min at 37°C. Then, the absorbance of the solution mixture was measured spectrophotometrically at 517nm. DPPH scavenging activity (%) was calculated using the following formula DPPH scavenging activity (%) = (Absorbance of a blank sample − Absorbance of the treated sample) / Absorbance of blank sample × 100

**Hydrogen peroxide scavenging activity**

Hydrogen peroxide scavenging activity of ESECC and biosynthesized ZnNP was measured using the method of ( Sudha et al., 2017).One millilitre of 0.1mM $H_2O_2$was taken in test tubes.ESECC, andZnNPextract were added at different concentrations 100µL, 200 µL, 400 µL, 800 µL, 1000 µL in each test tube. Then it was incubated for 15 minutes. After that reading was taken at 517nm using UV- Visible spectrophotometer. The absorbance was noted and the graph was plotted. The percentage inhibition was calculated using the formula Percentage Inhibition = Absorbance of control − Absorbance of test sample Absorbance of control × 100

**In vitro Anti- arthritic activity:**

**Xanthine oxidase inhibitory assay:**

The method of (Owens& John,1999) was followed for the xanthine oxidase inhibitory activity.Differentconcentrationsofplantethanolic extract and ZnNP loaded extractunderstudy in 0.15Mphosphatebuffer(pH7.5)and100µlof0.03U/ml xanthine enzyme were made up to 3.4 ml and incubated at 25°Cfor10min followed by the addition of 0.6mM xanthine substrate solution . After 10 minutes of incubation the formation of uric acid is estimated in the spectrophotometer. Allopurinol was used as a positive control.Thepercentage Xanthineoxidaseinhibitoryactivitiesofthe plant extracts werecalculated as follows Absorbance of control − Absorbance of test sample Absorbance of control × 100.

**Protein denaturation inhibition study:**

Method of (Mizushimaet al., 1968) was followed with minor modifications. The reaction mixture was consisting of ESECC and ZnNp extracts at different concentrations and 1% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of 1N HCl. acetyl salicylic acid was taken as standard drug. The samples were incubated at 37oC for 20 min and then heated at 57oC for 30 min. After cooling the samples, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate.Percent inhibition of protein denaturation was calculated as follows:
Percentage Inhibition = (A of control – A of sample)/A of control x100

**HRB membrane stabilization test**

HRB membrane stabilization test was performed by the following described method proposed by (Sadique et al.,1989).Fresh whole human blood (10ml) was collected and transferred to the centrifuge tubes. The tubes were centrifuged at 3000 rpm for 10min and were washed three times with equal volume of normal saline. The volume of blood was measured and re constituted as 10% v/v suspension with normal saline. The reaction mixture

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2ml consists of 1 ml of ESECC&ZnNP and 1 ml of 10% RBCs suspension, instead of test sample only saline was added to the control test tube acetyl salicylic acid was used as a standard drug. All the centrifuge tubes containing reaction mixture were incubated in water bath at 56ºC for 30min. At the end of the incubation the tubes were cooled under running tap water. The reaction mixture was centrifuged at 2500 rpm for 5 min and the absorbance of the supernatants was taken at 560 nm. The experiment was performed in triplicates for all the test samples. Percent membrane stabilization activity was calculated by the formulaPercentage Inhibition = (A of Control – A of Sample)/A of Control x100

***Insilico*Anti- arthritic activity**

**Molecular docking analysis**

*In silico* molecular docking studies were conducted with the help of patch dock to identify the best ligand for TLR2 and TLR4 proteins from the *Citrulluscolocynthis*stemPhyto compounds. The three-dimensional structures of toll-like receptor 4 (TLR-4) (PDB ID: 5NAO) and toll-like receptor 2 (TLR-2) (PDB ID:2Z80) proteins were downloaded from protein data bank (http://www.rcsb.org/pdb/). The two-dimensional structures for GC-MS identified 16 Phyto constituents from the *Citrullus colocynthis*were retrieved from PUB chemist, and converted into three-dimensional structures using Corina 3D converter. Using the patch dock server docking score regarding the ligand and protein interaction was identified. Using ligand showing appropriate docking score was selected and their further interactions with the proteins in terms of amino acid residues with the ligand and the active site were visualized and further confirmed using LIGPLOT. The docked results were saved as “pdb” file and binding affinity and molecular interaction between standard, test compounds and the receptor protein were visualized using PyMol Molecular Graphic System (Ver. 1.0) and Discovery Studio (Ver. 3.1) software, respectively the standard drug colchicine is used as a reference drug.

**Statistical Analysis**

The values reported are Mean±SE. The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnett’s’ test. The $< 0.05$ p values were considered as significant.

## Results:

### Characterization of*Citrulluscolocynthis*stem loaded zinc oxide nanoparticles (ESECC ZnNP)

Reduction of zinc oxide nanoparticles was initially identified by colour change from green colour to white in the reaction mixture at 1 hr incubation time. The colour change of reaction mixture was depending on the incubation time.

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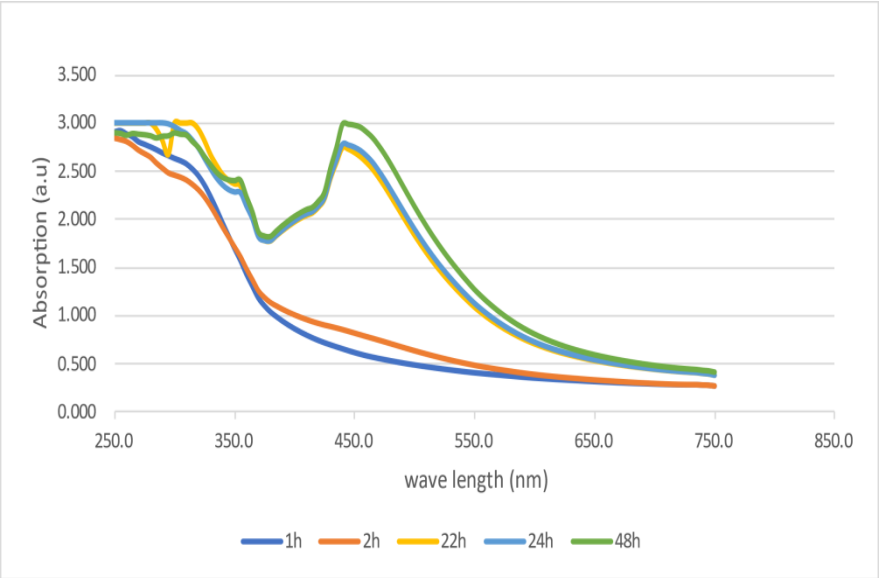


**Figure -1 characterization of ZnNP loaded *Citrulluscolocynthis* byUV-vis spectroscopy**

Reduction of zinc oxide nanoparticles was initially identified by colour change from green colour to white in the reaction mixture at 1 hr incubation time. The colour change of reaction mixture was depending on the incubation time. The deep white colour of biosynthesized zinc oxide nanoparticles was attained at 32 hr of incubation. Furthermore, the biosynthesis zinc nanoparticles from the *Citrullus colocynthis* extract was analysed by UV-vis spectroscopy at 200 to 700 Nano meters where the peak was clearly detected at300-750nm, which confirmed the formation of Zinc nano particles (Figure-1)

## Antioxidant activity

### DPPH scavenging activity

The DPPH scavenging activity of ESECC and ZnNP loaded with *Citrulluscolocynthis* was studied and depicted in Table-1. In the present investigation both the extracts revealed a concentration dependent increase in the scavenging activity. However, the ZnNP loaded *Citrulluscolocynthis* extract exhibited increased scavenging of DPPH when comparable (P>0.01) to the ethanolic extract of *Citrullus colocynthis*. Starting from lower concentrations the inhibitory activity ZnNP extract was similar with that of the positive control ascorbic acid used in the present investigation.

**Table-1 DPPH scavenging activity of different stem extracts of *Citrulluscolocynthis***

| Conc of Extract(in µg ) | % Inhibition of ESECC | % Inhibition of ESECCZnNP | % Inhibition of Ascorbic acid |
|---|---|---|---|
| 100 | 21.4±1.33 a ** | 32.76± 3.19 b ns | 35.12±2.7 |

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| | | | |
|---|---|---|---|
| 200 | 33.16±2.87 a** | 48.34±2.90 b ns | 50.23±3.2 |
| 400 | 42.15±2.05 a** | 56.45±1.15 b ns | 58.45±2.76 |
| 800 | 65.68±4.60 a ** | 69.15± 3.16 b ns | 72.2 ±2.15 |
| 1000 | 72.23±3.35 a ** | 79.90 ±4.20b ns | 82.16 ±2.49 |

Values are expressed in mean±SD (n=3), statistical significant test for comparison was done by ANOVA followed by Dunnet's 't' test.Comparison between a –Ascorbic acid vs ESECC b –Ascorbic acid vs ESECC ZnNP *p<0.05, **p<0.1 and ns – Non-Significant

**Hydrogen peroxide scavenging activity:**

The Hydrogen peroxide scavenging activity of ESECC and ZnNP loaded with *Citrulluscolocynthis* was shown in Figure-2. In the present investigation both the extracts revealed a concentration dependent increase in the scavenging activity. However, the ZnNp loaded *Citrullus colocynthis* extract exhibited increased scavenging of Hydrogen peroxide when comparable (P>0.01) to the ethanolic extract of *Citrullus colocynthis*. Starting from lower concentrations the inhibitory activity ZnNp extract was similar with that of the positive control ascorbic acid used in the present investigation.

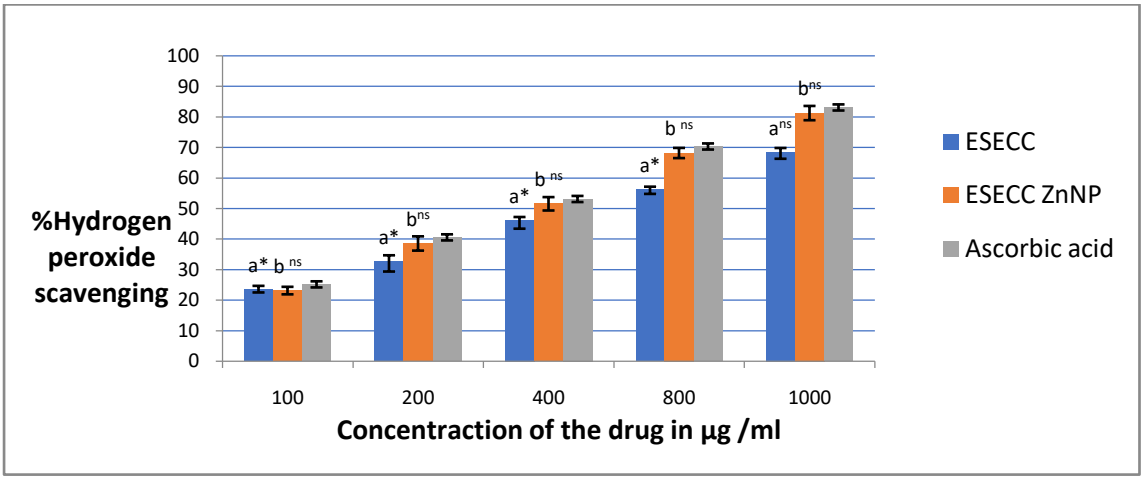


**Figure -2Hydrogen peroxide scavenging activity of different stem extracts of *Citrullus colocynthis***
Values are expressed in mean±SD (n=3), statistical significant test for comparison was done by ANOVA followed by Dunnet's 't' test.Comparison between a –Ascorbic acid vs ESECC b –Ascorbic acid vs ESECC ZnNP *p<0.05, **p<0.1 and ns – Non-Significant.

**Xanthine oxidase inhibitory activity:**

Figure-3 shows the inhibitory activity of xanthine oxidase by ESECC and ZnNP. In the present study the ZnNp extract of showed the higher inhibitory percentage when comparable

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to ethanolic extract. At the concentration of 800 and 1000µg/ml the inhibitory activity was quite similar to the positive drug Allopurinol.

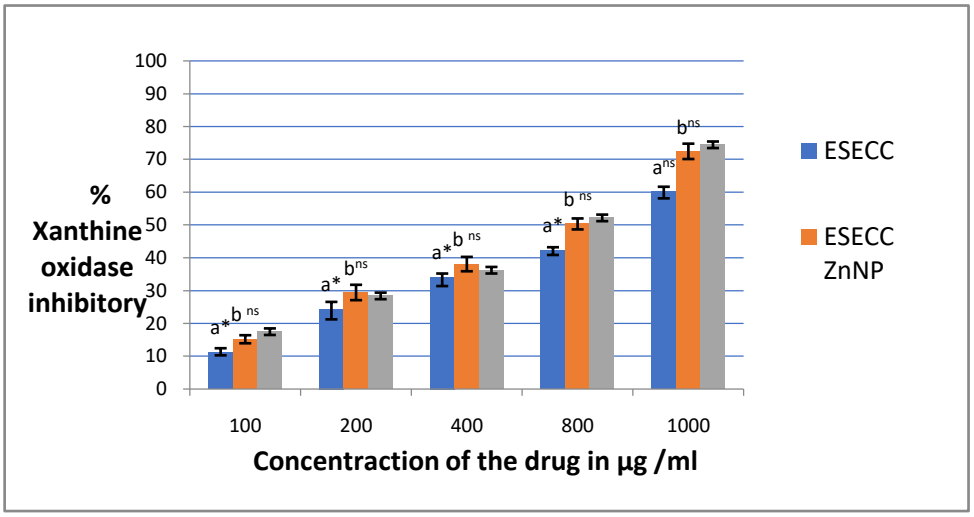


**Figure -3 Xanthine oxidase inhibitory activity of different stem extracts of *Citrulluscolocynthis***
Values are expressed in mean±SD (n=3), statistically significant test for comparison was done by ANOVA followed by Dunnet's 't'test.Comparison between a –Allopurinol vs ESECC b –Allopurinol vs ESECC ZnNP*p<0.05, **p<0.1 and ns – Non-Significant

## Protein denaturation inhibition study:

The inhibitory potential of protein denaturation was depicted in Table -2.Here also ESECC loaded ZnNP exhibited better inhibitory potential of protein denaturation than the ethanolic extract of *Citrulluscolocynthis*.

**Table-2 protein denaturation inhibition study of different stem extracts of *Citrulluscolocynthis***

| Conc of Extract (in µg ) | % Inhibition of ESECC | % Inhibition of ESECCZnNP | % Inhibition of Acetylsalicylic acid |
|---|---|---|---|
| 100 | 23.4±2.13 a ** | 28.56± 1.79 b ns | 29.42±4.17 |
| 200 | 34.78±1.70a** | 38.23±1.56 b ns | 40.59±2.20 |
| 400 | 41.76±3.75 a** | 46.65±3.05 b ns | 48.95±3.16 |
| 800 | 56.28±2.50 a ** | 64..79± 2.46 b ns | 66.80 ±3.45 |
| 1000 | 68.33±2.35 a ** | 78.55 ±3.20b ns | 80.76 ±2.09 |

Values are expressed in mean±SD (n=3), statistically significant test for comparison was done by ANOVA followed by Dunnet's 't' test.Comparison between a –Acetylsalicylic acid vs ESECC b –Acetylsalicylic acid vs ESECC ZnNP*p<0.05, **p<0.1 and ns – Non-Significant.

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Though a concentration dependent inhibitory activity is evident for both the extracts, at higher concentration of 1000µg/ml, the inhibitorypercentage was almost 78.55% against the positive control acetyl salicylic acid which showed 80.76% of inhibition.

**Membrane stabilization study:**

The protective effect of the plant extract on RBC membrane against the heat and hypotonic saline induced damage was shown in the Figure-4. There was a significant RBC membrane stabilityobserved in the ESECCZnNp treated group comparable ($p<0.01$) to theESECC treated group. This protection can be comparable to the acetyl salicylic acid treated group, since from concentration 800µg/ml the ZnNP and Acetyl salicylic acid groups exhibited almost similar type of protection in the present study.

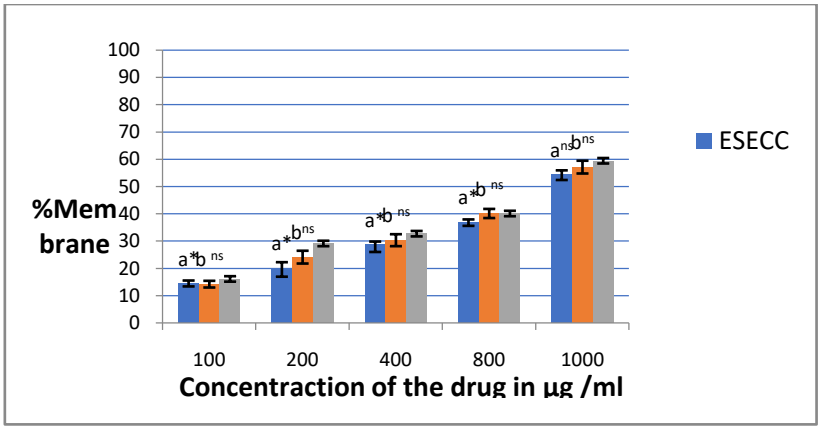


**Figure -4 Membrane stabilization activity of different stem extracts of *Citrullus colocynthis***

Values are expressed in mean±SD (n=3), statistically significant test for comparison was done by ANOVA followed by Dunnet's 't' test.Comparison between a –Acetylsalicylic acid vs ESECC b –Acetylsalicylic acid vs ESECC ZnNP *p<0.05, **p<0.1 and ns – Non-Significant.

## GCMS-Analysis

To identify the active ingredients present in the ethanolic extract of *Citrulluscolocynthis* GCMS analysis were performed and a total of 16 compounds were identified. Figure 5 and Table 3 enlists the phytochemicals identified in the chromatogram with their molecular formula,retentiontime and molecular weight.

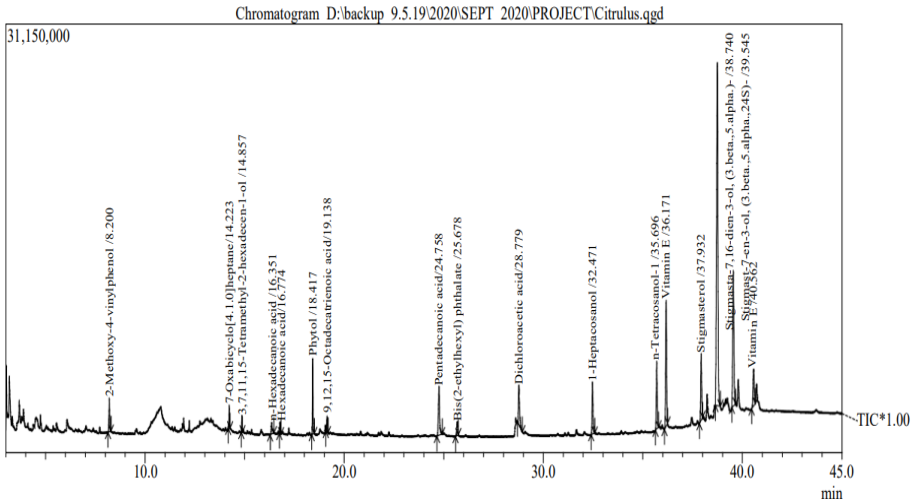


**Figure -5 GC-Ms Chromatogram of *Citrulluscolocynthis***

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**Table 3 GCMS analysis of ESECC:**

| Sl. No | RT | Name of the compound | Molecular Formula | Molecular Weight | Peak Area % |
|---|---|---|---|---|---|
| 1 | 8.200 | 2-Methoxy-4-Vinylphenol | $C_9H_{10}O_2$ | 150 | 2.14 |
| 2 | 14.223 | 7 -Oxabicyclic [4.1.0.] heptane | $C_{10}H_{16}O_2$ | 168 | 1.07 |
| 3 | 14.857 | 3,7,11,15-Tetramethyl-2-hexzdecen-1-ol | $C_{20}H_{40}O$ | 296 | 0.62 |
| 4 | 16.351 | n-Hexadecanoic acid | $C_{16}H_{32}O_2$ | 256 | 0.96 |
| 5 | 16.774 | Hexadecanoic acid | $C_{18}H_{36}O_2$ | 284 | 0.55 |
| 6 | 18.417 | Phytol | $C_{20}H_{40}O$ | 296 | 0.96 |
| 7 | 19.138 | 9,12,15-Octadecatrienoic acid | $C_{20}H_{34}O_2$ | 306 | 0.79 |
| 8 | 24.758 | Pentadecanoic acid | $C_{18}H_{36}O_4$ | 316 | 4.84 |
| 9 | 25.678 | Bis (2-ethylhexyl phthalate | $C_{24}H_{38}O_4$ | 390 | 1.11 |
| 10 | 28.779 | Dichloroacetic acid | $C_{15}H_{24}Cl_2O_2$ | 306 | 3.65 |
| 11 | 32.471 | 1-Heptacosanol | $C_{27}H_{56}O$ | 396 | 4.04 |
| 12 | 35.696 | n-Tetracosanol-1 | $C_{29}H_{50}O_2$ | 354 | 5.24 |
| 13 | 36.171 | Vitamin E | $C_{29}H_{50}O_2$ | 430 | 9.91 |
| 14 | 37.932 | Stigmasterol | $C_{29}H_{48}O$ | 412 | 5.37 |
| 15 | 38.740 | Stigmasta-7,16-dien-3-0l (bet,5alpha) | $C_{29}H_{48}O$ | 412 | 38.99 |
| 16 | 39.545 | Stigmast-7-en-3-ol, (beta,5, alpha.,24s) | :$C_{29}H_{50}O$ | 414 | 13.76 |

## Insilico Anti gout activity

The *Insilico* anti gout activity was evaluated in terms of the inhibitory activity of the proteins TLR2 and TLR4,since they are responsible for triggering the inflammatory reaction against the monosodium urate crystals leading to gouty arthritis. The ligands Methoxy-4-vinylphenol,Pentadecanoic acid andPhytol were selected as a best antagonistic ligand based on the docking score among the 16 ligands identified in the GCMs analysis. The Table 4 explains the docking score values of the compounds interaction with TLR2 and TLR4 proteins by means of patch dock analysis

**Table -4 Docking score values of compounds with TLR2and TLR4**

| S.NO | Compound Name | Docking score | |
|---|---|---|---|
| | | TLR2 | TLR4 |
| 1 | 2-Methoxy-4-Vinylphenol | **-3.5** | **-4.2** |
| 2 | 7 -Oxabicyclic [4.1.0.] heptane | -4.5 | -4.7 |
| 3 | 3,7,11,15-Tetramethyl-2-hexzdecen-1-ol | -5.1 | -5.3 |
| 4 | n-Hexadecanoic acid | -6.8 | -6.3 |
| 5 | Hexadecanoic acid | -4.8 | -4.3 |
| 6 | Phytol | **-2.2** | **-3.1** |
| 7 | 9,12,15-Octadecatrienoic acid | -5.8 | -6.3 |
| 8 | Pentadecanoic acid | **-3.8** | **-3.1** |
| 9 | Bis (2-ethylhexyl phthalate | -4.8 | -4.3 |
| 10 | Dichloroacetic acid | -4.7 | -4.3 |
| 11 | 1-Heptacosanol | -5.4 | -6.3 |
| 12 | n-Tetracosanol-1 | -4.7 | -5.3 |
| 13 | Vitamin E | -6.3 | -5.3 |
| 14 | Stigmasterol | -5.7 | -7.3 |
| 15 | Stigmasta-7,16-dien-3-0l (bet,5alpha) | -7.8 | -7.3 |

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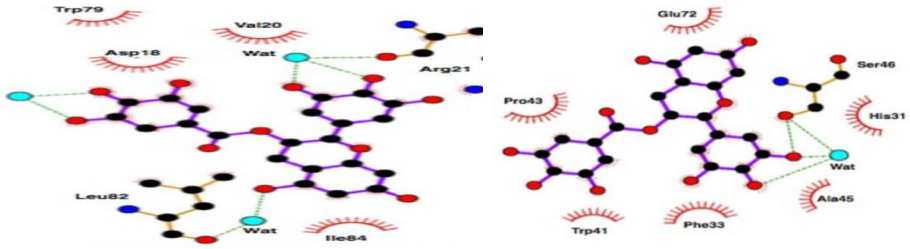
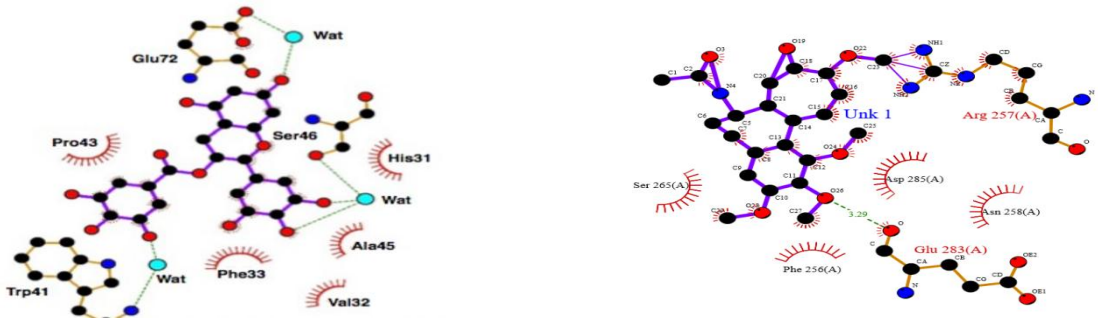
| | | | |
|---|---|---|---|
| 16 | Stigmast-7-en-3-ol, (beta,5, alpha.,24s) | -8.8 | -8.3 |

Based on their docking score these three ligands were further evaluated for the inhibitory interaction in terms of their amino acid interaction with phyto ligands ( Figure6 and 7) The positive drug colchicine is also docked in the present study.

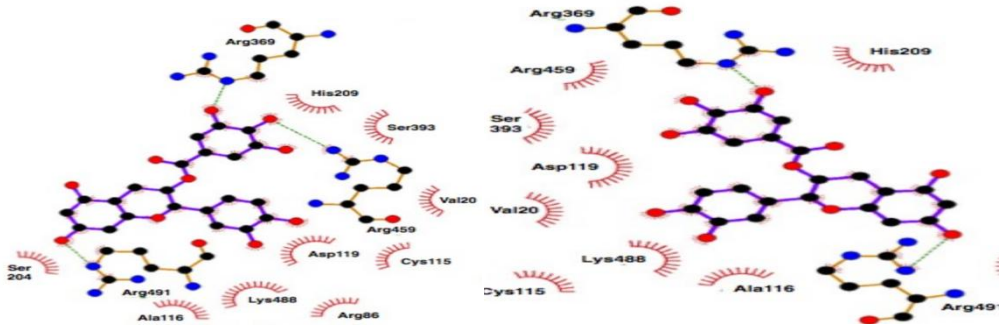



**(A) 2-Methoxy-4-Vinylphenol** **(B) Pentadecanoic acid**




**(C) Phytol(D) colchicines**

**Figure: 6Interaction of the phyto ligands with Amino acids of TLR-2 protein**




**(A) 2-Methoxy-4-Vinylphenol(B) Pentadecanoic acid**

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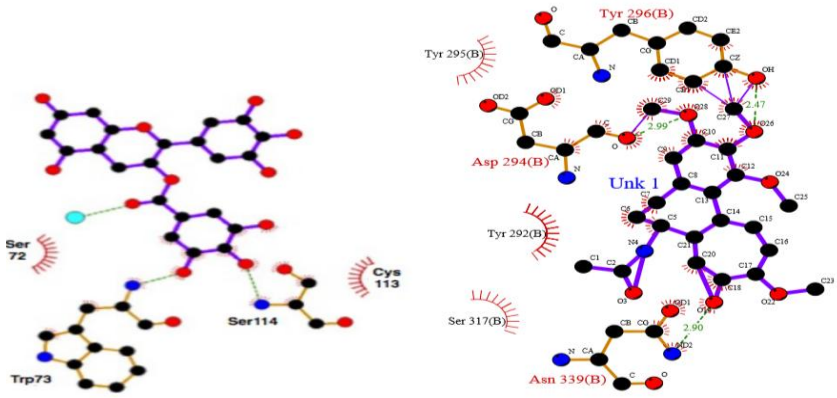


**(C) Phytol(D) colchicines**

**Figure: 7Interaction of the phyto ligands with Amino acids of TLR-4 protein**

It is observed in the present study that the phyto ligands methylvinyphenol, pentadecanoic acid and phytol,have the ability to interact with the arginine aminoacid present in these two proteins and there by contribute towards their antagonistic nature. Similarly serine amino acid is inhibited by the ligand phytol which act in the similar way as that of the colchicine the positive drug used in the presentstudy.

**Discussion:**

In gouty arthritis, the deposition of MSU crystals in the articular tissues triggers the immune response in the neutrophils and initiates a variety of molecular events which leads to pain and inflammation. It is mandatory to follow two treatment strategies for gouty arthritis. Initially, the drug should be capable of reducing hyperuricemia by inhibiting the enzyme xanthine oxidase and secondly it should reduce the immune response to reduce inflammation, pain and protect the bones and tissues from auto destruction. In the present investigation comparable to the ESECC and ZnNp, the drug exhibited a dose dependent inhibitory activity of xanthine oxidase enzyme. The researchers (Arokiyarajet al., 2008) have reported the presence of terpenoids, phenol, gums, flavonoids, saponins, sugars, alkaloids and steroids in the ethanolic extract could be responsible for the xanthine oxidase enzyme inhibitory activity that is observed in the present study. In the present research, we evaluated the ability of our extracts in terms of its inhibition of protein denaturation, membrane stabilization and proteinase inhibition since these are the common cause of inflammation. synovial proliferation and joint tissue destruction which occurs in gouty arthritis also occur(Meyer et al.,2005).

The ZnNp extract exhibits a better anti gout arthritic activity when compared to ESECC in the present study. Upon the recognition of the MSU crystals, the neutrophils infiltration occurs which is followed by the release of the lysosomal enzymes in the form of proteases leading to further inflammation and injury to the cell membrane. The lysosomal membrane mimics the RBC membrane and the substances that protect the RBC membrane and inhibit the haemolysis will also protect the lysosomal membrane and prevent the release of inflammatory mediators, mainly the protein degrading enzymes. Additionally, injury to the

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lysosomal membrane initiates the secretion of phospholipase A2 (PLA2) which, in turn, digests phospholipids to release the fatty acid arachidonic acid (Bieber& Terkeltaub,2004)

Arachidonic acid which is the substrate for the enzyme cyclo oxygenase produces the prostaglandins which is one of the inflammatory mediators and a pain causing agent in any type of tissue injury (Choi et al.,2004). In the present research, the ZnNp extract exhibited, not only membrane stabilizing effect but also inhibits protease enzymes and protein denaturation. Significant analgesic activity may be expected for the plant extract by the inhibition and the release of phospholipase A2 and thereby the synthesis of prostaglandin is also halted. The bioactive substances Stachydrine, 3-hydroxyl stachydrine, cad bine**,** terpenoids and flavones present in the ZnNp extract specifically inhibit these inflammatory mediators has the protective effect as an anti-gout agent(Akashi et al ., 2007)

Complex inflammatory reactions play a predominant role in any type of arthritis, which ultimately induces the immune system to produce several mediators, which will cause destruction of cells and severe pain leading to disability(Sherwood et al., 2004). A potential drug should act in a multifaceted manner to treat this chronic pain causing and disabling disease. So in the present research, by *Insilco* methods, we analysed whether the Phyto-constituents present in the stem extract of *Citrullus colocynthis* act as an antagonist ligand to the enzyme the TLR 2 and TLR4 and thereby reduces the pain and tissue destruction. Based on the Ligplot analysis, three components namely methylvinyphenol, pentadecanoic acid and phytolserves as a better antagonistic ligand in inhibiting the above enzymes thereby protecting the joints from pain and destruction.

**Conclusion:**

Thesuccessfultreatment for gouty arthritis requires the management of pain and joint destruction as the consequence of the complex immunochemical reactions against the MSU deposition in the joints. The ZnNP extract of *Citrullus colocynthis* contain several Phyto constituents which have the ability to inhibit these immune mediated inflammatory reactions as evidenced in the present study. So, this plant extract can be used as a potential drug for the treatment of gouty arthritis.

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