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Pharmacological potential of nickel nanoparticles using *Tribulus Terrestris* plant extracts (Stem)

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Abstract
In recent years, green synthesis of nanoparticles NiNPs has gained much interest from chemists and researchers. In this concern, Indian flora has yet to divulge innumerable sources of cost–effective non-hazardous reducing and stabilizing compounds utilized in preparing NiNPs. This study investigates an efficient and sustainable route of NiNPs preparation from 1mM aqueous NiNPs using stem extracts of plant like Tribulus terrestris well adored for their wide availability and medicinal property. NiNPs obtained showed significantly higher anti-oxidant by DPPH scavenging assay, Anti- inflammatory activity by Inhibition of Albumen Denaturation and Anti diabetic activity by Inhibition of Alpha-Amylase In totality, the NiNPs prepared are safe to be discharged in the environment and possibility utilized in processes of pollution remediation. NiNPs may also be efficienctly utilized in agricultural research to obtain better health of crop plants as shown by our study.

Keywords: green synthesis, NiNPs, anti-oxidant anti-inflammatory etc

Introduction

The genus *Tribulus*, belonging to family Zygophyllaceae, comprises about 20 species in the world, of which three species, viz. *Tribulus cistoides*, *Tribulus terrestris*, and Tribulus alatus, are of common occurrence in India [1]. Among them, *T. terrestris* (TT) is a well-patronized medicinal herb by Ayurvedic seers as well as by modern herbalists [2]. The plant is used individually as a single therapeutic agent or as a prime or subordinate component of many compound formulations and food supplements. It is an annual shrub found in Mediterranean, subtropical, and desert climate regions around the world, viz. India, China, southern USA, Mexico, Spain, and Bulgaria [3, 4].

Fig 1: Tribulus terrestris

In addition to all these applications, the Ayurvedic Pharmacopoeia of India attributes cardiotonic properties to the root and fruit. In traditional Chinese medicine, the fruits were used in eye trouble, edema, abdominal distension, emission, morbid leukorrhea and sexual dysfunction. Shern-Nong Pharmacopoeia (the oldest known pharmacological work in China) described its use in depressed liver, mastitis, flatulence, acute conjunctivitis, headache and vitiligo. In Unani medicine, TT is used as diuretic, mild laxative and as a general tonic [5], his plant is a most important ingredient of an Ayurvedic preparation. Leaf decoction is used as mouth gargle. Leaves increase the menstrual flow and cure gonorrhea. The root is claimed to be stomachic, appetizer, diuretic and carminative. It is used in well-known ayurvedic medicines namely Gokshuradi *Guggul*, *Dashmoolarishtha* and *Amritha Prasa Ghritha* prescribed for several diseases [6].

Saponin from TT possesses hypoglycemic properties [7]. TT significantly reduced the level of serum glucose, serum triglyceride, and serum cholesterol, while serum superoxide dismutase (SOD) activity was found to be increased in alloxan-induced diabetic mice. The decoction of TT showed inhibition of gluconeogenesis in mice [8, 9]. TT ethanolic extract at 2 g/kg body weight produced protective effect in streptozotocin-induced diabetic rats by inhibiting oxidative stress. Ethanolic extract of TT exhibited 70% inhibition of α-glucosidase at 500 µg/ml using maltose as the substrate and 100% inhibition of aldose reductase at a dose of 30 µg/ml using dl-glyceraldehyde as the substrate [10].

The ethanolic extract of TT inhibited the expression of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) in lipopolysaccharide-stimulated RAW264.7 cells. It also suppressed the expression of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-4 in macrophage cell line. Thus, the ethanolic extract of TT inhibits the

expression of mediators related to inflammation and expression of inflammatory cytokines, which has a beneficial effect on various inflammatory conditions [11]. The methanolic extract of TT showed a dose-dependent inhibition of rat paw volume in carrageenan-induced inflammation in rats [12].

An ethanolic extract of TT fruits was tested in urolithiasis induced by glass bead implantation in albino rats by Anand *et al*. It exhibited significant dose-dependent protection against deposition of calculogenic material around the glass bead, leukocytosis, and elevation in serum urea levels. Subsequent fractionation of the ethanol extract led to decrease in activity [13]. Various other biochemical parameters in urine, serum, and the histopathology of urinary bladder were restored in a dose-dependent manner. A novel antilithic protein having cytoprotective potency and of molecular weight ~ 60 kDa was purified from TT [14]. Aggarwal tested the activity of TT on the nucleation and growth of calcium oxalate (CaOx) crystals as well as on oxalate-induced cell injury of NRK 52E renal epithelial cells. The experiments revealed that TT extract not only has a potential to inhibit nucleation and growth of the CaOx crystals but also has a cytoprotective role [14]. TT was found to inhibit stone formation in various models of urolithiasis using sodium glycolate and ethylene glycol [15].

Materials and Methods

Collection of root

Fresh root of the samples were collected from Perambalur, during the month of December.

Preparation of root extract

The fresh and young root of the samples were collected & washed thoroughly with sterile double distilled water (DDW). Twenty grams of sterilized root samples were taken and cut into small pieces. Finely cut roots were placed in a 500 ml Erlenmeyer flask containing 50ml of sterile DDW. After that, the mixture was transferred to Soxhlet apparatus to derive extracts. The extract was stored in 4 ^{0}C.

Microwave assisted synthesis of metal nanoparticles.

Metal nitrate was used as precursor in the synthesis of metal nanoparticles**.** 100 ml of root extract was added to 100 ml of 0.1N metal nitrate aqueous solution in conical flask of 250 ml content at room temperature. The flask was thereafter put into shaker (100 rpm) at 50^0 C and reaction was carried out for a period of 12 hrs. Then the mixture is kept in microwave oven for exposure of heat. Metals nanoparticles are made by a chemical reduction of a metal salt in the presence of a stabilizing agent. Rapid microwave heating and agitation gives mono dispersed particles. Add 200 ml of extract with 1M metal nitrate in beaker and Cover loosely. Expose the sample in Microwave radiation for 20 minutes at 100% power. The setting of time is done on the basis of trial and error method. The color will continue to change with respect to time. The mixture was completely dried after a period of 20 minutes and hence nano particles in form of powders were obtained.

Anti-Oxidant Studies

DPPH scavenging assay

The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method of Mensor *et al*., (2001). *Reagents* 2, 2-Diphenyl-1-picryl hydrazyl (DPPH) – 90.25mM in methanol in a dark room.

Procedure

To a methanolic solution of DPPH (90.25 mM), an equal volume of ethanolic Rhizome of Cyperus rotundus L (250-1500 µg) was added and made up to 1.0 mL with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Systronics UV-visible Spectrophotometer. Ascorbic acid was used as standard for comparison. The inhibition of free radicals by DPPH in percentage terms (%) was calculated by using the following equation.

$$\% \text{ Scavenging} = \frac{\text{A Control OD - A sample}}{\text{A blank5}} \times 100$$

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

Scavenging of H_2O_2

The ability of the Cyperus rotundus L to scavenge H_2O_2 was determined according to the method of Ruch *et al*., (1989).

Reagents:

1. Phosphate buffer (pH 7.4)
2. H_2O_2 solution (0.6 mL, 40 mM)

Procedure:

A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). H_2O_2 concentration was determined spectro photo metrically from absorption at 230 nm in a spectrophotometer (SL 159, UV- Visible Spec, Elico, India). Extracts (200, 400, 600, 800 and 1000 µg) in distilled water were added to a H_2O_2 solution (0.6 mL, 40 mM). Absorbance of H_2O_2 at 230 nm was determined after ten minute against a blank solution containing phosphate buffer without H_2O_2. The percentage of scavenging of H_2O_2 of Cyperus rotundus Land standard was calculated using the following equation:

$$\% \text{ Scavenging} = \frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

Anti- inflammatory activity:

Inhibition of Albumen Denaturation

Method as prescribed (Sakat *et al*., 2010) was followed with modifications. The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectro photo metrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100.

Inhibition of Alpha-Amylase Enzyme

Starch solution (0.1% w/v) was prepared by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3, 5-di nitro salicylic acid solution 96 mM. The starch solution is added to the both control and plants extract tubes and left to react with α-amylase solution, under alkaline conditions at 25ºC. The reaction was allowed for 3 min. The generation of maltose was quantified by the reduction of 3, 5-dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540 nm (Malik and Singh 1980).

$$\% \text{ Inhibition} = \frac{\text{Control OD - Test OD}}{\text{Control OD}} \times 100$$

Results and Discussions

Anti-oxidant

DPPH scavenging assay

There are several methods available to assess the antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant screening of plant extracts. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases.

In the present study, the Nickel nanoparticles using Root extracts of *Tribulus terrestris* have high DPPH scavenging capacity, which increased with increasing concentration [Table 1 and Figure 1] The DPPH assay was carried out at different concentrations of Nickel nanoparticles using stem extracts of *Tribulus terrestris* samples, namely 250 mg/ml, 500 mg/ml, 750 mg/ml and 1000 mg/ml. DPPH assay did not show any significant difference at 250 mg/ml Concentrations in *Tribulus terrestris*, however, it was significant for 500 mg/ml and 750 mg/ml and 1000 mg/ml for the nanoparticles, all the values are compared with standard drug of Ascorbic acid. DPPH is a relatively stable free radical. DPPH radical react with suitable reducing agents, the electrons become paired off, and the solution losses color stoichiometrically depending on the number of electrons taken up. Hence, this assay provided information on the reactivity of test samples with a stable free radical. The decrease in the absorbance of the DPPH radical caused by test samples was due to the scavenging of radical by electron donation.

Table 1: Anti-Oxidant activity of Nickel nano particles using stem extracts of Tribulus terrestris by DPPH Scavenging assay activity.

Test	Concentration of the sample (mg/ml)	250	500	750	1000
DPPH	% of inhibition of the TT	13.09	36	47.5	51.02
	Ascorbic acid (Standard)	23.63	29	46.25	52.05

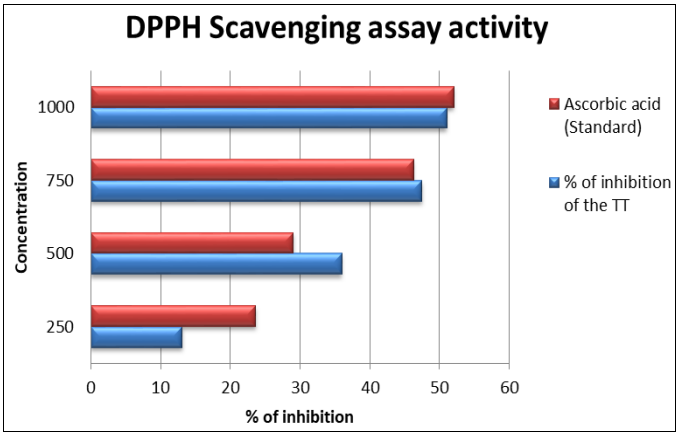

Fig 2: Graphical Representation of Anti-Oxidant activity of Nickel nano particles using stem extracts of Tribulus terrestris by DPPH Scavenging assay activity.

Anti- inflammatory activity

Inhibition of Albumen Denaturation

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study, the protein denaturation bioassay was selected for *in vitro* assessment of the anti-inflammatory property of Nickel nanoparticles synthesized *Tribulus terrestris*. The Albumen Denaturation is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein. The mechanism of denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding. In the presence study, denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Selected extracts were effective in inhibiting heat-induced albumin denaturation. Aspirin was used as a standard antiinflammation drug as shown in Figure [Table 2 and Figure 2]. The albumin denaturation method was carried out at different concentrations of Cobalt nanoparticles using Root extracts of *Tribulus terrestris* samples, albumin denaturation, 100µg/ml 200µg/ml, 300 µg/ml, 400 µg/ml and500 µg/ml. Albumen Denaturation did not show any significant difference at 100 mg/ml Concentrations in *Tribulus terrestris*, however, it was significant for 200-500 mg/ml for the nanoparticles, all the values are compared with standard drug of Aspirin.

Table 2: Anti Inflammatory activity Inhibition of Nickel nano particles using stem extracts of Tribulus terrestris by of Albumen Denaturation activity.

Test	Concentration of the sample (mg/ml)	100	200	300	400	500
Albumin denaturation	% of inhibition of the TT	44	54	57.3	62.3	70.3
	Aspirin (Standard)	45	56.25	66.2	72.02	82

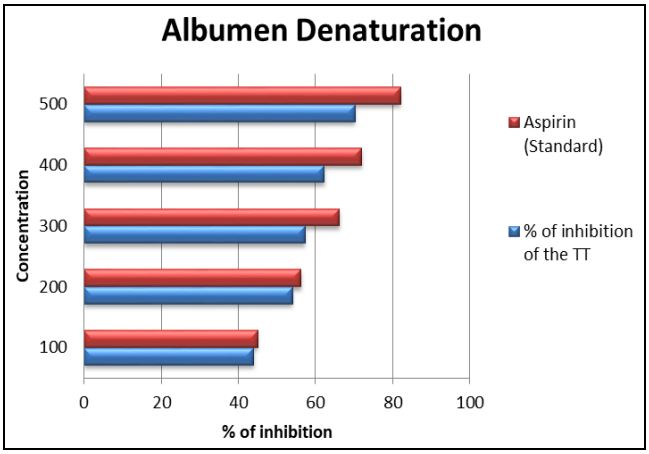

Fig 3: Graphical Representation of Anti Inflammatory activity of Nickel nano particles using stem extracts of Tribulus terrestris by Albumen Denaturation activity.

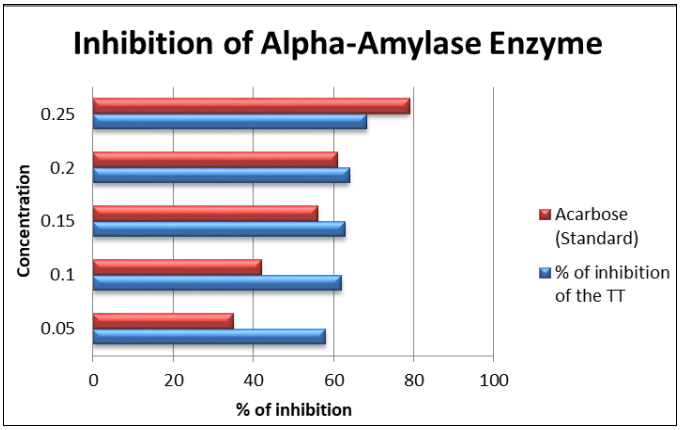

Fig 4: Graphical Representation of Anti diabetic activity of Nickel nano particles using stem extracts of Tribulus terrestris by Inhibition of Alpha-Amylase Enzyme activity.

Antidiabetic activity

Inhibition of Alpha-Amylase Enzyme

Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. A therapeutic approach to decrease the hyperglycaemia is to inhibit the carbohydrate digesting enzymes (α-glucosidase and α-amylase), thereby preventing the breakdown of carbohydrates into monosaccharides which is a main cause of increasing blood glucose level. Therefore, developing compounds having inhibitory activities towards carbohydrate hydrolysing enzymes may be a useful way to manage diabetes. As shown in Figure 3 and Table 3, α-amylase and α-glucosidase were significantly inhibited in a dose-dependent manner by the NiNPs. The results suggest that with the increased NiNPs concentration, the activity levels of enzyme were remarkably reduced, Hence, the biomolecules likely enhanced the antidiabetic potential of the synthesized NPs. α-Amylase inhibitory actions were observed in increasing order, as Acarbose (Figure 3). Comparable results were observed. However, the foregoing results suggest that the synthesized NiNPs are potentially better antidiabetic particles at inhibiting carbohydrate digesting enzymes, and could prove an effective approach in the diabetes care.

The Alpha-Amylase Enzymewas carried out at different concentrations of Cobalt nanoparticles using Root extracts of *Tribulus terrestris* samples, namely Alpha-Amylase Enzyme 0.5 µg/ml, 0.10µg/ml, 0.15µg/ml, 0.20µg/ml and 0.25 µg/ml.Albumen Denaturation did not show any significant difference at 0.5 µg/ml and 0.10µg/ml, Concentrations in *Tribulus terrestris*, however, it was significant for 0.15µg/ml, 0.20µg/ml and 0.25 µg/ml for the nanoparticles, all the values are compared with standard drug of Acarbose. Figure 3. Antidiabetic activity of synthesized NiNPs based on inhibition of α-amylase and activity.

Table 3: Anti diabetic activity of Nickel nano particles using stem extracts of Tribulus terrestris by Inhibition of Alpha-Amylase Enzyme activity.

Test	Concentration of the sample (mg/ml)	0.05	0.1	0.15	0.2	0.25
Alpha amylase inhibitory activity	% of inhibition of the TT	58.03	62.06	63.02	64	68.3
	Acarbose (Standard)	35	42	56	61	79

Conclusion

Present studies are reported that,

Antioxidant activity is defined "as a limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage in oxidative chain reactions" and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radical.

The antioxidant activity of synthesized NiNPs were determined by using DPPH assay. DPPH is a stable compound which can be reduced by accepting the hydrogen or electrons and has been widely used to evaluate the antioxidant activity. The effect of different concentrations of NiNPs on DPPH radical antioxidant activity is shown in Table 1. Our results revealed that the aqueous fruit extract and synthesized NiNPs are free radical scavengers. However, the NiNPs exhibited more scavenging activity of DPPH than aqueous Root extract. The DPPH activity of the NiNPs were found to increase in adose-dependent manner.

The ability of the synthesized Nickel nanoparticles to inhibit albumin denaturation has been investigated for potential anti-inflammatory action mechanism. It is well documented that protein denaturation is involved in arthritic reactions and development of tissue damage during inflammation. Results reveal that synthesized Nickel nanoparticles were effective in inhibiting thermally induced albumin denaturation at all tested concentrations, indicating their capability of controlling protein denaturation involved in the inflammatory process. Upon translating from *in vitro* to *in vivo* systems, Carrageenan-induced rat hind paw edema model was used to assess the anti-inflammatory potential of Nickel nanoparticles loaded from *Tribulus terrestris* stem extract. It is a widely used experimental model of acute inflammation that exhibits a high degree of reproducibility. The characteristic swelling that occurs in the rat paw is due to increased vascular permeability and edema formation. This result suggests that nanoparticles consisting of Nickel may interfere with the release of acute inflammatory mediators or antagonize their action. Thus, the inhibition of edema formation and albumin denaturation activities of Nickel nanoparticles from *Tribulus terrestris* aqueous extract clearly establish their anti-inflammatory potential and therefore could be considered as potential source of the anti-inflammatory drug. However, one should try to further figure out other action mechanisms responsible for this activity via other detailed experimentations.

NiNPs synthesized from Root extract of *Tribulus terrestris*were effectively inhibited carbohydrate-hydrolyzing enzyme i.e. α-amylase. With increasing of NiNPs concentration, the enzymatic activity level was significantly induced. NiNPs inhibition of α-amylase were *Tribulus terrestris*is shown in (Fig.3) and it was revealed with previous literature for green synthesis of NiNPs using *Tribulus terrestris stem* extract having potent *in vitro* antidiabetic activity were reported.More potent *in vitro* antidiabetic activity to the small size and presence of bioreduction capping agents.

Green synthesis of NiNPs could significantly overcome the problem of using chemical agents that cause various adverse effects. Therefore, green synthesis of NPs is environment-friendly approach. Nickel nanoparticles using stem extracts of *Tribulus terrestris* plant possess pharmacological activity that is, considerably enhanced after incorporation with NPs

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