

# Synthesis, Characterization and Bioactivity Profiling of Gold Nanoparticles of *Trachyspermum ammi* Crude Extract

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## Abstract

*Trachyspermum ammi* seeds were selected for photochemical study. The crude *T. ammi* methanol and aqueous extracts showed the presence of alkaloids, saponins, steroids, terpenoids, coumarins, betacyanins, flavonoids and soluble starch. The synthesis of gold nanoparticles (AuNPs) using *T. ammi* extract was characterized using UV-visible, and FT-IR spectroscopy. The appearance of sharp peak at 520 nm in the UV visible spectra, and the appearance of broad band nanoparticles spectra at 563.21  $\text{cm}^{-1}$ , 516.92  $\text{cm}^{-1}$  and 462.92  $\text{cm}^{-1}$  as well as the disappearance of the carboxyl OH bond and carbon-carbon triple bond supported the formation of AuNPs. The crude *T. ammi* methanol and AuNPs were investigated for antioxidant potential using DPPH· free radical assay, which shows that crude extract has significant antioxidant effect. The synthesized AuNPs was also evaluated for antibacterial activities against *staphylococcus aureus*, *Klebsiella pneumonia* and *Bacillus subtilis*. The crude extract showed activity against *Bacillus subtilis*, while AuNPs showed activity against *staphylococcus aureus*. The *in vivo* sedative effect and analgesic effect were enhanced in AuNPs treated animals in 5 times less dose (i.e., 5, and 10 mg/kg) than that of crude extract. It was concluded that *T. ammi* extract includes capping and reducing agents, which make it capable to be developed as stable AuNPs. The biological action of AuNPs is either enhanced (sedative and analgesic) or changed (antibacterial activity), when compared with that of plant extract.

**Keywords:** Nanotechnology, phytochemicals, biological activities, sedative effect, analgesic effect

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## INTRODUCTION

The medicinal plants provided the ailments and cure throughout human history. Medicinal plants are capable of making various ranges of secondary metabolites which possess diverse biological potentials<sup>1</sup>. Even at dawn of 21<sup>st</sup> century, about 90% of probable novel drug molecules have been purified directly or indirectly<sup>2</sup>. As per WHO report about 80% of the people of some Africo-Asian countries nowadays, use herbal medicine for some aspect of primary health care<sup>3</sup>. The clinical application of herbal drugs faces the same challenges as allopathic medicines like effectiveness, drug delivery, safety, selectivity, solubility, toxicity and frequent dosing<sup>4,5</sup>. The modern pharmaceutical research could overcome the abovementioned challenges by adopting novel drug delivery techniques for herbal medicines, such as development of matrix system, micro emulsion, solid dispersion, liposomes and solid lipid NPs, nanoparticles (NPs)<sup>6,7</sup>.

The field of nanotechnology has grown widely in past few decades with efficient applications in biomedical sciences, food processing, and pharmacology<sup>8-10</sup>. It deals with matter at the scale of one billionth of a meter, so the NPs is the most fundamental component in the fabrication of a nanostructure. The nano-size atoms and molecules exhibit different properties, and ultimately show a variety of surprising and interesting results<sup>11,12</sup>. There are various methods reported for fabrication of NPs, but metal NPs like gold and silver are extensively studied due to their unique electrical and optical properties. Gold nanoparticles (AuNPs) are increasingly studied because of their wide applications in antibacterial agents, detectors, and sensors<sup>13-15</sup>. In old Indian medical system (Ayurveda), gold salt was used as medicine in the preparations to treat anemia, tuberculosis, and cough. In traditional medicines, the gold has been explored for various disease ailment and shown with efficient antibacterial and antifungal properties<sup>16-19</sup>.

*Trachyspermum ammi* is an annual herbal plant belongs to family Apiaceae, indigenous to Egypt but cultivated in Iran, Pakistan and India and Europe. It is commonly known as Ajwain in India. The green seeds as well as fruits *T. ammi* are well recognized for medical and nutritional purposes<sup>20</sup>. The structural composition of *T. ammi*

include proteins (17.1%), fats (21.1%), glycosides (12%), carbohydrates, saponins, flavones and other components (7.1%) involving copper, calcium, cobalt, iron, iodine, manganese, nicotinic acid, phosphorous, riboflavin, and thiamine. The *T. ammi* is known for its brownish essential oil (5%) that responsible for its odor and taste<sup>21</sup>. The plant has medical application in Medieval and Traditional Persian Medicine for paralysis, tremor and palsy as well as other disorders in the field of neurology. The plant used for the auditory and olfactory weakness and infections<sup>20,21</sup>. The *T. ammi* was beneficial in cough, Pleurisy and dysphonia, anthelmintic, gastrointestinal disorders and have carminative properties. The Persian practitioner considered the seeds as a diuretic agent and beneficial for dissolving the stones when used with wine<sup>22</sup>.

In the present study, we aimed to synthesize the AuNPs of *T. ammi* methanol and aqueous extract keeping in view its extensive medical applications. The synthesized AuNPs were characterized and analyzed for its stability. The newly synthesized AuNPs were biologically screened analgesic, sedative and antibacterial effects and compared with respective therapeutic effect of the crude *T. ammi* extract.

## MATERIALS AND METHODS

### Chemicals and plant sampling

Gold chloride, potassium bromide, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, sulphuric acid, and trichloro methane were from Sigma (St. Louis, MO, USA). *Trachyspermum ammi* was procured from local market (Peshawar, Pakistan). The plant was identified by taxonomist Ghulam Jilani from Botany Department, University of Peshawar, Pakistan.

### Extraction of *T. ammi* seeds

To prepare the extract, 500 g of *T. ammi* seeds were dipped in distilled water and methanol using conical flask, respectively and kept in water bath. The two solutions, obtained after three days, were filtered and the filtrates were concentrated in rotary evaporator. Further evaporation was allowed in water bath for two days to procure water and methanol solid extract<sup>21,22</sup>.

### Phytochemicals profiling

Tests for phytochemicals like tannins, anthraquinones, alkaloids, saponins, glycosides,

reducing sugars, flavonoids, steroids, terpenoids, phlobatanins, anthocyanins, betacyanins, cardiac glycosides, emodins, coumarins and free reducing sugars were carried out<sup>20-22</sup>.

### Synthesis of AuNPs

#### Preparation of stock and gold salt solution

The extract stock solution was prepared by dissolving 1 g of solid extracts of *T. ammi* (methanolic and aqueous) in 100 mL methanol and/or water. Another stock solution of gold salt was prepared by adding 34 mg of gold chloride to 100 mL of methanol solution<sup>18</sup>.

#### Formation of AuNPs

Four samples of 5 mL stock solution of water and methanol extracts were added to the 5 mL, 10 mL, 15 mL, and 20 mL gold salts solution separately, stirred on hot stirring plates on 40 to 60°C for 60 min and observed its UV spectrum for confirmation of synthesis of AuNPs<sup>18</sup>.

#### Characterization of AuNPs

##### UV visible spectroscopy

UV visible spectra was recorded at room temperature to monitor the formation and stability of AuNPs. The wave length ( $\lambda$ ) were fixed between 300 nm and 700 nm and the base line was drawn by the methanol solution. The peak in the 500-600 nm range indicates the presence of AuNPs<sup>23</sup>.

##### FTIR Spectroscopy

An FTIR spectrum was recorded and the measurement was performed to recognize the possible functional groups of molecules responsible for capping and effective stabilization of the nanoparticles synthesized<sup>18</sup>.

#### Biological evaluation of crude extract and AuNPs

The crude extract and synthesized AuNPs were evaluated *in vitro* and *in vivo* for various biological activities.

##### *In vitro* biological activities

###### Antibacterial activity

Antibacterial activity was performed by disk diffusion technique. The procedure was done in laminar airflow, Forceps, *Petri* dish, cotton swab, disc sterilized and utilized for plating. Sterile environment was maintained by HEPA (high performance efficient particle air) in the laminar flow. A few colonies of the organism are inoculated in 5 mL broth and cultured for 2 h. The cultures were diluted to a density of the 1 % barium sulfate standard. After 15 min bacterial

cultures were spread on agar surface by spread plating method. When the inoculum dried, the impregnated discs were spread on the agar surface with flamed forceps and pressed down to ensure contact. The discs were sterilized by autoclaving and then dried at 80°C for at least 1 h. The sterile discs were immersed in extract solution (2 mg/mL), and AuNPs solution (1 mg/mL) then placed on *Petri* plates. The impregnated discs are dried for 5 min and washed with water. The discs dried at 25°C for 15 min were pressed down to make certain complete contact of the disc with the agar surface. Ampicillin was used as positive control for antibacterial screening test. The discs were spread out far sufficient to stop both reflection waves from the preparation of methanolic extract edges of the *Petri* plates and overlapping rings of inhibition. The plates were incubated at 37°C for 12 h. The diameter of the zone of inhibition was determined<sup>19</sup>.

###### Antioxidant activity

The stock solution of crude extract was organized by dissolving 25 mg in 50 mL methanol. For antioxidant activity, different concentrations were prepared from stock solution by diluting with methanol (i.e., 20, 40, 60, 80, 100 and 150  $\mu$ g/mL). Similar concentrations were prepared for AuNPs. One mL of DPPH $\cdot$  was added to each of PPM concentration and the solution was placed in the darkness for 30 min and then UV-visible spectra was measured at 515 nm. Antioxidant activity of both crude and nanoparticles base line is made on a solution of 4 mL methanol and 1 mL DPPH $\cdot$  and it's percent activity is calculated by the following formula<sup>24</sup>:

$$\text{Percent (\% activity) = control-absorbance / control*100}$$

##### *In vivo* biological activities

###### Animals

In this experiment BALB/c mice were used. Mice were kept under standard light/dark cycles and temperature with standard supply of food (normal laboratory food and water *ad-libitum*). Prior to the experiment, mice were familiarized with laboratory conditions. The rulings of the institutes of Laboratory Animal Resources, Commission on Life Sciences, National Research Council were maintained during whole course of study. The protocols of all experiments

were approved by the ethical committee of the Department of Pharmacy, University of Peshawar, Pakistan.

#### Acute toxicity testing

The acute toxicity screening was done as per reported procedure of Khan *et al.* Animals were divided into various groups ( $n=6$ ). The extract of *T. ammi* was screened at 50, 100, 200, 300, 400, 500 mg/kg (body weight of mice). The AuNPs of *T. ammi* was screened at 1, 5, 10, 20, 30, 40 mg/kg (body weight mice). After administration (i.p.) of the above-mentioned test doses, the animals were observed for 24 h duration. The mortality and survival ratio was noted and percent mortality was calculated<sup>25</sup>.

#### Sedative activity

The design of the device for sedative activity covers an area of whitish woody structure possessing a diameter of 1500 mm. The stainless steel was used for fencing and the structure was partitioned into 19 square boxes. Open field of the structure was positioned in a room with ample space. Balb-C mice ( $n=6$ ) weighing  $26 \pm 4$  g were used. The mice were familiarized with light of red color by keeping them under a 40 Watt red bulb for 1 h prior to start of the experiment. The normal control animals were injected distilled water as blank (10 mL/kg). The reference group was treated with 0.5 mg/kg diazepam. The rest of the groups were treated with crude extract (50, and 100 mg/kg, i.p.) and AuNPs (5 and 10 mg/kg, i.p.). After a time interval of 30 min, all mice were placed one by one in the middle of box, and the number of lines passed by each animal was noted<sup>25</sup>.

#### Acetic acid induced writhing test

Peripheral nociceptive action of *T. ammi* extracts along with synthesized AuNPs was examined by applying the acetic acid-induced writhing test<sup>25</sup>. The pre-screened mice were distributed in 6 groups where each group contained six animals. The pain was triggered by injecting 0.9% acetic acid (v/v, 0.1 mL/10 g body weight) intraperitoneally. The control group (six animals) was given normal saline (10 mL/kg, i.p.), whereas second group was provided with diclofenac sodium (10 mg/kg i.p.) as a standard drug. The remaining groups received *Trachyspermum ammi* extract (50 and 100 mg/kg i.p.) and synthesized AuNPs (5 and 10 mg/kg i.p.). After the injection of 0.9% acetic

acid, the total numbers of contractions in muscles were recorded for 20 minutes each group and compared to that of control group (saline treated group).

#### Statistical analysis

All of the results are presented as mean  $\pm$  S.E.M and One-way ANOVA was applied in order to compare significant variances among groups followed by Dunnet's multiple comparison post-test. The significance level ( $p < 0.05$  or  $0.01$ ) was considered valid in the study. The results of antibacterial and antioxidant activities are also given as mean  $\pm$  SEM of three readings and their statistical analysis were carried out using the GraphPad program.

## RESULTS AND DISCUSSION

#### Phytochemical screening

The analysis of *T. ammi* aqueous and methanol extract exhibited the presence of various phytochemicals (Table 1). The aqueous extract of *T. ammi* showed positive result for the presence of alkaloids, flavonoids, terpenoids, and saponins. Methanol crude extract showed positive results for the presence of flavonoids, betacyanins, terpenoids, and steroids. This preliminary phytochemical

**Table 1.** Phytochemical assortment of *Trachyspermum ammi* extracts

Chemical components	Aqueous extract	Methanol extract
Alkaloids	+	-
Tannins	-	-
Anthraquinones	-	-
Glycosides	-	-
Reducing Sugars	-	-
Saponins	+	-
Flavonoids	+	+
Phlobatannins	-	-
Steroids	-	+
Terpenoids	+	+
Cardiac glycosides	-	-
Coumarin	-	+
Emodins	-	-
Betacyanins	-	+
Carbohydrates	-	-
Monosaccharides	-	-
Free reducing sugars	-	-
Combined reducing sugars	-	-
Starch	-	-

screening made it clear that the both extracts have various phytochemicals which would act as both capping and reducing agents for the synthesis of NPs<sup>20-22</sup>.

### Characterization of AuNPs

#### UV-visible spectroscopy

The production of AuNPs is generally detected by UV-Vis spectroscopy which specifically measures surface plasmon resonance peaks of AuNPs. The noble metals exhibited distinctive optical properties due to the property of surface plasmon resonance. When *T. ammi* extract was added to salt solution at 40-60°C temperature, the solutions changed from yellow to brown, indicating the formation of AuNPs. The color of the solution was changed because of reduction of gold ( $\text{Au}^{3+}$  to  $\text{Au}^0$ ) by the active phytochemicals present in *T. ammi* extract<sup>26</sup>. Previously reported studies showed that phytochemicals act as capping and reducing agents. The variety of constituents in the natural extracts leading to the synthesis of symmetrical NPs<sup>27</sup>. Experiment was carried out with varying stock solutions of *T. ammi* extract and salt concentrations. In order to monitor the formation and stability of AuNPs, the absorption spectra of the synthesized AuNPs were recorded

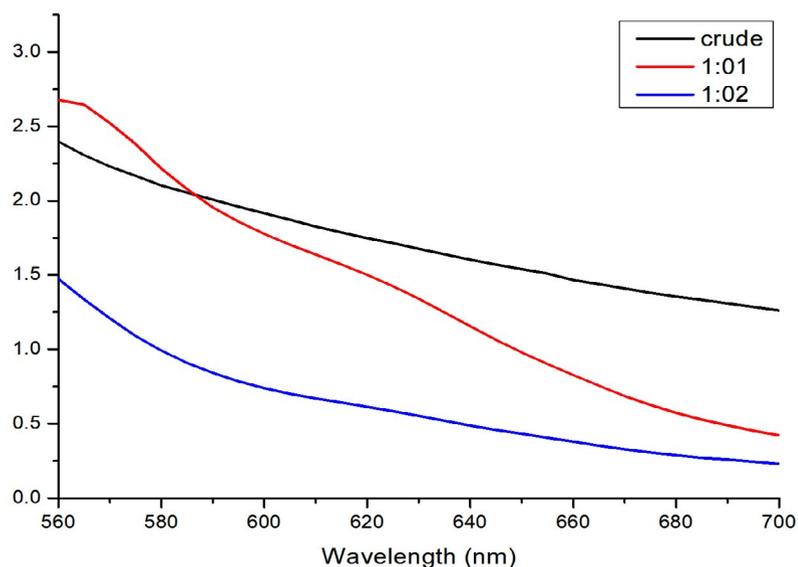
against methanol. The peak absorbance of AuNPs was observed in wavelength ( $\lambda$ ) range of 500-600 nm in methanol solutions, which exhibited the successful formation of AuNPs.

#### FTIR spectra analysis of crude extract and AuNPs

The FTIR spectra acquired from the crude extract of *T. ammi* and synthesized AuNPs are demonstrated in Fig. 2 and 3. In Fig. 2, The O-H bonds stretching is attributed by a broader peak observed at  $3392\text{ cm}^{-1}$ , evidenced the presence of aromatic alcoholic and phenolic compounds. The peaks such as  $2947.23\text{ cm}^{-1}$ ,  $2831.50\text{ cm}^{-1}$ ,  $2592.33\text{ cm}^{-1}$ , and  $2522.89\text{ cm}^{-1}$  reflect carboxylic acid bond

**Table 2.** DPPH• free radical scavenging activities of *T. ammi* crude extract and AuNPs

Concen. ( $\mu\text{g/mL}$ )	Percent activity (%)	
	Methanol extract	AuNPs
20	44.79	36.40
40	58.91	46.97
60	64.51	54.56
80	76.34	60.16
100	82.17	61.60
150	84.22	63.59



**Fig. 1.** UV-visible spectra of *T. ammi* extract solution and synthesized AuNPs

Where,

Crude: extract solution of *T. ammi*,

1:01: showed AuNPs synthesized by mixing stock solution and salt solution (1:1, v/v),

1:02: showed AuNPs synthesized by mixing stock solution and salt solution with (1:2, v/v)

stretching. The peak at  $2368.59\text{ cm}^{-1}$ ,  $2222.00\text{ cm}^{-1}$  and  $2044.54\text{ cm}^{-1}$  show  $\text{C}\equiv\text{C}$  stretching. The peak of  $1658.78\text{ cm}^{-1}$  shows carbonyl group and peak at  $1411.89\text{ cm}^{-1}$  shows C-C stretching. The peak at  $1211.30\text{ cm}^{-1}$  shows C-N stretching and peak at  $1026.13\text{ cm}^{-1}$  shows C-H single bond stretching. The peak at  $655.80\text{ cm}^{-1}$  shows C-Br stretching.

When this FTIR spectra was compared with that of AuNPs, the spectra exhibited different IR absorption. The FTIR spectra acquired for AuNPs after 1 hour of reaction for observing the involved functional groups present in *T. ammi* extract (Fig. 3). The peaks at  $2592.33\text{ cm}^{-1}$ ,  $2522.89\text{ cm}^{-1}$ ,  $2368.59\text{ cm}^{-1}$  and  $2222.00\text{ cm}^{-1}$  disappear in the spectra of FTIR spectra of AuNPs. Thus, it means that carboxyl bond and carbon-carbon triple are considered accountable for reduction and stabilization of AuNPs<sup>28</sup>. Broad bands observed in spectra of AuNPs  $563.21\text{ cm}^{-1}$ ,  $515.92\text{ cm}^{-1}$  and  $462.92\text{ cm}^{-1}$  validated the formation of AuNPs, which were not observed in the crude FTIR Spectrum<sup>29</sup>.

#### Biological evaluation of crude extract and AuNPs Antioxidant Activity

The free radical scavenging against DPPH is a simple and commonly used assay for the study

of antioxidant potential of tested compounds<sup>30-32</sup>. This is a chromophoric assay in which change in absorbance is indicated by change in color and then measured. The free scavenging effect of crude extract vs synthesized AuNPs is shown in the Table 2. The antioxidant activity of crude extract of *T. ammi* was higher as compared to the AuNPs. As the concentration of the stock solution and AuNPs increases, its antioxidant effect increases. The data demonstrated that the net antioxidant effect of AuNPs decrease as compared to extract.

#### Antibacterial activity

To analyze the comparative antibacterial effect of crude extract and AuNPs, they were screened against gram positive and negative bacteria as given in Table 3. The crude extract as well as synthesized AuNPs were ineffective against *Klebsiella pneumonia*. The crude extract exhibited sample shows activity for *Bacillus subtilis*, while AuNPs showed activity for *staphylococcus aureus*. However in both cases the zone of inhibition (10mm) was far less than that of standard ampicillin (30mm). The data exhibited that the AuNPs synthesis of *T. ammi* extract changed its antibacterial properties. The thymol isolated from *T.ammi* is potent antibacterial agent and

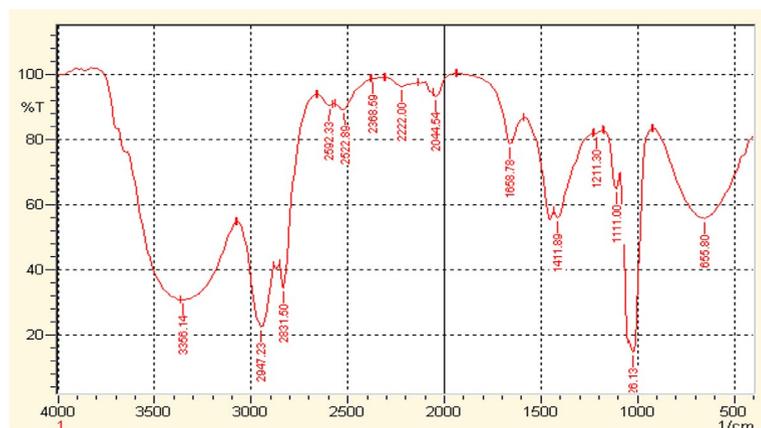


Fig. 2. FTIR spectrum of crude *T. ammi* extract

Table 3. Antibacterial activity of *T. ammi* crude extract and AuNPs

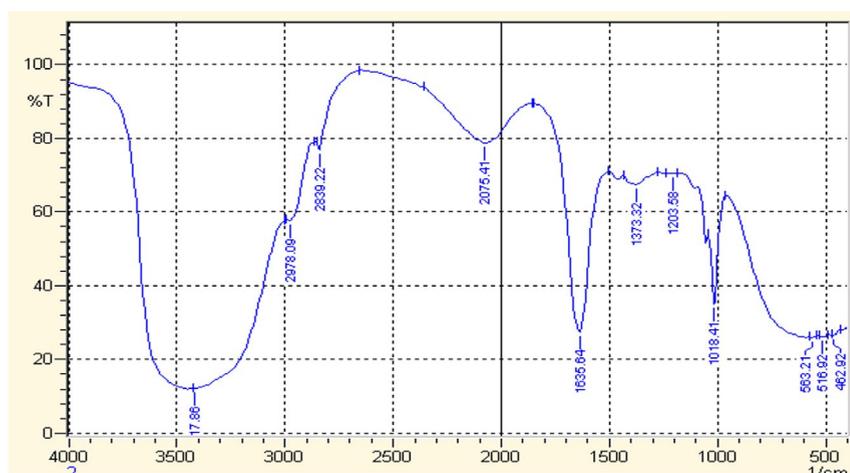
Microorganism	Gram stain	Diameter of zone of inhibition (mm)		
		Crude extract	AuNPs	Ampicillin
<i>Klebsiella Pneumonia</i>	-	0	0	30
<i>Staphylococcus aureus</i>	+	0	10	30
<i>Bacillus subtilis</i>	+	10	0	30

**Table 4.** Sedative activity of *Trachyspermum ammi* extract and AuNPs

Treatment	Dose (mg/kg)	No. of lines crossed in 10 min
Distilled water	10	125±1.15
Diazepam	0.5	6 ± 0.14
Extract	50	122.4±2.70
	100	113.4 ±2.42
AuNPs nanoparticles	5	96.56±0.84
	10	82.40±1.78

**Table 5.** Analgesic activity of *Trachyspermum ammi* extract and AuNPs

Treatment	Dose (mg/kg)	No. of lines crossed in 10 min
Normal saline	10 (mL/kg)	65.4±3.48
Diclofenac sodium	1	17.6±1.38
Extract	50	52.3±2.44
	100	30.2±2.13
AuNPs	5	48.2±2.00
	10	26.3±1.67

**Fig. 3.** FTIR spectrum of AuNPs synthesized by mixing extract stock solution and salt solution (1:2, v/v)

considered to disrupt bacterial membrane function<sup>34</sup>.

#### Acute toxicity dose determination

The acute toxicity of *T. ammi* crude extract was assessed in dose of 50, 100, 200, 300, 400, and 500 mg/kg. Whereas the AuNPs was screened at doses of 1, 5, 10, 20, 30, and 40 mg/kg. The animals exhibited behavioral toxicity, no mortality during 24 h of assessment. For the comparison of *in vivo* effect assessment between extract and AuNPs, extract dose used was 50, and 100 mg/kg, however, AuNPs dose used was 5-times less than that of extract (i.e., 5 and 10 mg/kg).

#### Sedative activity

The sedative effect of natural products is frequently assessed by application of open field assay in experimental animals<sup>33</sup>. By comparing the treated (with extract and AuNPs) groups with diazepam-treated group, it was found that *T. ammi* has very high potential for sedation. Pretreatment

of mice with *T. ammi* crude extract (50 mg/kg) showed locomotory activity comparable to normal animal, however, slight decrease was observed at dose of 100 mg/kg. Pretreatment of mice with AuNPs (5, and 10 mg/kg) showed locomotory activity comparable with that of high doses of extract (50, and 100 mg/kg). The data showed that the effect of AuNPs enhanced compared to *T. ammi* extract. The sedative potential of *T. ammi* has already been explored<sup>34</sup>. Various compounds have tendency to change neurological behavior<sup>35</sup>. The monoterpene (thymol) isolated from *T. ammi* is evaluated as allosteric modulator of GABAA receptor<sup>36</sup>. The same GABA receptor is the site of action for reference drug diazepam thereby increasing the chloride influx and causes neuronal hyperpolarization.

#### Analgesic activity

Various methods are applied for analyzing the peripheral or central analgesic potential of plant extracts and drugs. One of most preferred

method for assessment of peripheral analgesia is the acetic acid induced pain model, due to its sensitivity, rapid execution<sup>37</sup>. The method is based on the principle of analyzing abdominal writhes (constriction) produced by inflammatory markers after acetic acid injection. The substances producing inhibition of writhing may associate with decline production or inhibition of inflammatory markers, such as prostaglandins, prostacyclins, in the decreased production or inhibition of proteinoids, at peripheral level<sup>38,39</sup>. The substances capable of inhibition of receptors for the inflammatory markers could exhibit the peripheral analgesic effect. The analgesic effect of active constituents present in samples under analysis is measured in terms of inhibition of acetic acid induced abdominal constrictions (writhing). Therefore, we evaluated the analgesic potential of extract and AuNPs by using acetic acid induced writhing test. Our data showed that *T. ammi* extract in 50 mg/kg has mild analgesic potential, which increased to moderate effect at dose of 100 mg/kg compared to reference drug. Slight enhanced analgesic activity was observed in less doses of AuNPs (5 to 10 mg/kg). The analgesic and anti-inflammatory potential of *T. ammi* has already been explored in various studies<sup>34,40</sup>. The *T. ammi* extract contain terpenes, sterols and glycosides, which are suggested to have effect on kinin, bradykinin, prostaglandins and lysozymes synthesis thereby reducing inflammation and allodyna (pain)<sup>41,42</sup>.

## CONCLUSION

The data concluded that the *T. ammi* extract have necessary capping and reducing agents, which make it capable to be developed as stable AuNPs. The AuNPs showed enhanced biological effect which explain that the pharmacokinetic parameters like bioavailability is increased. The biological action of AuNPs is enhanced in case of antibacterial activity, which means that AuNPs might change the specificity of *T. ammi* and likewise other drugs.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

SB, AR, HN, SUAS, JA, GU, and F drafted the manuscript, compiled information from the literature, and designed the Fig. and tables. SB, AR, HN, SUAS, JA, GU, and F drafted the manuscript and gathered information from the literature. MFR reviewed the manuscript.

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## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

## ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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