Original Article

Phytochemical Constituents and In vitro Pharmacological Response of *Cnidium monnieri;* A Natural Ancient Medicinal Herb

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Abstract

Background: Natural medicines are being used for the treatment of various disorders due to pharmacological, therapeutical, and nutraceuticals characteristics.

Objectives: Current research was planned to explore In vitro pharmacological response of phytochemical constituents extracted from *C. monnieri*' seeds using aqueous ethanol (70%).

Methods: Qualitative and quantitative measurements for phytochemical constituents were performed following reference protocols. Then In vitro antioxidant potential, cytotoxic studies, antimicrobial, and spermicidal pharmacological response of *C. monnieri* extract were investigated.

Results: The results of High Performance Liquid Chromatography (HPLC), Fourier Transform Infra-Red (FTIR) spectroscopy, and Atomic Absorption Spectrophotometer (AAS) explored the presence of wide range of bioactive compounds with significant ($p<.05$) antioxidant activities. Cytotoxic studies revealed significant ($p<.05$) protective behavior of *C. monnieri* evaluated using CtDNA damage protection, against *Salmonella typhi* TA98 and TA100, RBCs membrane stabilizing and clot lysis assay. It was also found that selected herb has antibacterial and antifungal activities. The results of spermicidal study on human (n = 30) spermatozoa revealed significant ($p<.05$) contraceptive per vaginal behavior of this natural medicinal plant.

Conclusion: It could be concluded that *C. monnieri* showed significant pharmacological activities with non-toxic behavior, however In vivo study in animals and clinical trials are required to declare this natural herb as therapeutic agent.

Keywords

Natural medicine, phytochemical constituents, pharmacological response, In vitro, contraceptive

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Introduction

Since earlier civilizations, medicinal plants have been used by human being for their survival and growth.[1] WHO (2007) had estimated that the use of conventional treatments in developing countries for primary health care is about 80% and involves the use of different extracts obtained from herbal plants.[2] Even though pharmaceutical drugs are employed for treating many infectious as well as non-infectious diseases, but they are very costly with several side effects, thus it is important to seek alternative appropriate sources to resolve these problems. In this case, healthy and organic foods obtained from natural plants having abundant phytochemicals can be a hope to provide balanced diet for a growing population around the world. Oxidative damage may cause a wide range of contagious diseases like diabetes, obesity, aging, cancer, cardiovascular diseases, joint disorders, and Alzheimer's disease.[3] Phenolic compounds such as polyphenols, found in medicinal plants play a major role in pharmacological and many biological activities like antioxidants, anti-allergic, antimicrobial, anti-inflammatory, cardioprotective, anticarcinogenic, and vasodilatory effects. Many plants with medicinal values are rich with active metabolites, flavonoids, phenolic acids and terpenoids. These active plant metabolites are useful in the scavenging of free radicals, metal chelating and the reduction of single tone oxygen.[4,5]

C. monnieri (L. Cuss.) belongs to the family of Umbelliferas and is commonly used as a traditional herbal medicine for the treatment of various illnesses in China, Japan, and Vietnam. This is a yearly plant commonly known as Xà Sàng Tù in Vietnam, "She Chuang Zi" in China and "Jashoshi" in Japan. *C. monnieri* (L.) Cuss has been identified to contain almost 350 phytochemical components like bornyl isovalerate, alpha-pinene, isoborneol, cnidiline, cnidimine (edultin), osthol (coumarin), isopimpinellin, columbianadin, isopimpelline, imperatorin, xanthotoxol, archangelicin, glucoside, bergaptene, xanthotoxin (cnidimonal), and sesquiterpenes. Compounds such as osthole and coumarin have been identified as the active ingredients responsible for the pharmacological effects, although mechanism of action of *C. monnieri* is still unknown. Studies are further needed to analyze the relationship of structure-activity and to expose the toxicity and clinical consequences of the plant before being used as a pharmaceutical agent.[6]

C. monnieri (L.) have been reported to have a variety of therapeutic properties including female genitals health, cure male impotence, deal with antipruritic, treat skin problems, exhibited strong anti-allergic, antimicrobial, and treating osteoporosis.[7] These pharmacological properties of this natural medicinal plant might be due to its strong antioxidant characteristics. Taking all these aspects into consideration, this research aimed to track the therapeutic effects of this traditional medicinal plant by evaluating In vitro antioxidant, cytotoxic, antimicrobial, and spermicidal pharmacological response of *C. monnieri* hydroethanolic extract.

Material and Method

Selection and Collection of Plant Material

The seeds of *C. monnieri* were identified and authenticated taxonomically from Department of Botany, Government College University, Faisalabad-Pakistan (Ref#Bot-2019-7794) after purchasing from the Local market of Faisalabad-Pakistan. Then, washed seeds were powdered using electronic grinder (Model CB 222, Cambridge, UK) after drying in shade at room temperature and in oven set at 50°C for overnight. Hydroethanolic (30:70 v/v) extract was prepare following the protocol of Sulaiman et al.,[8] with some modification.

Phytochemical Analysis

As phytochemical screening procedure, qualitative analysis for phytochemical constituents including steroids, flavonoids, alkaloids, glycosides, triterpenoids, tannins, and saponins was performed following the standard methods.[9]

Quantitative Analysis

Total phenolic contents (TPC) and Total flavonoids contents (TFC)

Total phenolic contents (TPC) estimation in Hydroethanolic extract was done following the Folin-Ciocalteu method as described by Jain et al.[9] Total flavonoids contents (TFC) were quantified using the protocol described by Pranuthi et al.

High Performance Liquid Chromatography (HPLC) for phenolic compounds

High Performance Liquid Chromatography (HPLC) (C_{18} column having 250 × 4.6 mm internal diameter with 5 µm film thickness, accompanying an oven set at 30°C) was used to determine the selected phenolic compounds in the extract following the method as described by Yue et al.,[11] with minor modifications. Chromera HPLC system (Perkin Elmer, USA.) attached with Flexer Binary LC pump, UV/Vis LC Detector (Shelton CT, 06 484 USA) controlled by software V. 4.2. 6410 used to analyze the data. Acetonitrile: methanol (70: 30) as solvent A and double distilled water having glacial acetic acid (.5%) solvent B in mobile phase were used. Many phytochemical compounds were identified using 275 nm wavelength using standards to compare the retention times and spiking.

Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectrophotometer to explore the various functional chemical linkages in the extracted phytochemicals was used. Munir et al.,[12] protocol was followed using FTIR spectrometer (Model Bruker Platinum ATR with accessories A225/Q Platinum ATR Multiple Crystals CRY diamond and having Interferogram Size of 10 550 points) in the frequency range of 400–4000/cm available in the Central Hi-Tech Laboratory, Government College University, Faisalabad-Pakistan was used.

Trace Elements and Heavy Metals Estimation

Nitric-perchloric acid method, Colagar et al.,[13] protocol was used to digest the seeds for measurement of trace and heavy metals (ppm) using atomic absorption spectrophotometer (AAS) (Aurora, Canada) available in Central Hi-Tech Laboratory, Government College University, Faisalabad-Pakistan. Cu, Fe, Cd, Pb, Mg, Co, Zn, Ni, and Mn as acted important bio elements were measured by AAS.

Investigations of Antioxidant Potential of C. monnieri Extract Using Different Assays

Total Antioxidant Capacity (TAC) (Phosphomolybdenum Method). TAC of the hydroethanolic extract was estimated by Phosphomolybdenum assay, a spectrophotometric method, following the protocol of Prieto et al. Ascorbic acid (25, 50, 100, 150, 200, 250, and 300 µg/ml) in methanol (absolute) was used as standard for the construction of standard curve. Butylated hydroxytoluene (BHT) was used as reference controls.

2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

The antioxidant capacity of herb extract against Vitamin C and BHT as standard using DPPH radical scavenging assay was determined following Jain et al.,[9] protocol. Only methanolic DPPH solution as blank was used to calculate as

$$\text{DPPH Inhibition (\%)} = \textit{Blank abs } (A_0) - \textit{Sample abs } (A_1)/\textit{Blank abs } (A_0) \times 100$$

Hydrogen Peroxide (H_2O_2) Scavenging potential. Jain et al.,[9] method was used to measure the H_2O_2 scavenging potential of the seeds extract. Vitamin C as standard and PBS as blank were used taking absorbance at 230 nm and percent hydrogen peroxide scavenging capacity was calculated as

$$\%\ \text{Scavenged}\ [H_2O_2] = \frac{[1 - \text{AS}]}{\text{AS}}\ \text{x}\ 100$$

where AS = absorbance in the presence of the extract sample or standard.

Reducing power Assay (FRAP method). For the measurement of antioxidant potential of selected herb ferric reducing power assay (FRAP) was used as described by Pranuthi et al.,[10] in which the reducing potential of substance Fe^{3+} $(CN)_6$ into Fe^{2+} $(CN)_6$ by direct electron donation was measured using 25, 50, 100, 150, 200, 250, 300, 350, and 400 (µg/mL) concentrations of extract in, respectively, labeled tests tubes.

In vitro Cytotoxic studies of C. monnieri Extract

Cytotoxic Potential by Hemolytic Assay. To evaluate the cytotoxic status of hydroethanolic plant extract hemolytic assay was used following the protocol of Munir et al.,[12] with some modifications using RBCs suspension (7.0×10^8 RBCs/mL) in triplicates and calculated as

$$\text{Percent Hemolysis} = \{\text{Ae} - \text{Ap}/\text{Ad} - \text{Ap}\} \times 100$$

Here Ae = the absorbance of plant extract; Ap = the absorbance of PBS; Ad = the absorbance of DMSO (20%) used to make plants dilution.

Thrombolytic Potential by Clot Lysis Assay

To investigate the clot dissolving potential of selected natural herb, Munir et al.,[12] protocol was used. Healthy volunteers (n = 10) for the collection of fresh venous blood (1 mL) (excluded those having any transmittable infection or taking any type of anticoagulant) were recruited as instructed by Institutional Research Scrutiny Committee (Ref. No. GCUF/DAS/19/1534). PBS as negative control while streptokinase vial (1 500 000 IU) as positive control were used, and percentage clot dissolving potential calculated as

$$\text{Clot Dissolving activity}(\%) = \frac{(\text{clot Initial weight} - \text{clot final weight})}{\text{clot initial weight}} \times 100$$

Ames Test (Mutagenicity/Genotoxicity evaluation)

The Bacterial Reverse Mutation Test (Ames test) developed by Bruce Ames in 1970s used as a screening method in drug development because of its simplicity and relatively low cost to prelude genotoxic impact of medicines before clinical usage.[15] *Salmonella typhimurium* two strains TA98 and TA100 as auxotrophic bacterial strains were used to evaluate the genotoxicity through reverse mutations using fluctuation method on incubating for up to 5 days in 96 well microplates. Probability test was performed to evaluate the results statistically.

Calf Thymus DNA Damage Prevention Test

Calf thymus DNA (Ct DNA) was used to explore the genoprotective ability of aqueous ethanolic extract of *C. monnieri* following the method of Munir et al.,[12] with some modifications. Fenton reagent composed of 30% (v/v) hydrogen peroxide and ferrous sulphate (2 mM) as DNA damage inducer was incubated with Ct DNA and natural herb extract (100 µg/mL), respectively. Agarose gel electrophoresis was used to compare the DNA damage protection capacity of selected plant extract along with controls DNA and gel documentation was done by Syngene GeneGenius Gel Light Imaging System.

Antibacterial and Antifungal Potential of C. monnieri' Hydroethanolic Extract

For the evaluation of antimicrobial activities, pre identified bacterial strains including *Bacillus subtilis, Staphylococcus aureus, Pasteurella multocida, Escherichia coli, Klebsiella pneumoniae, Acinetobacter* species, *Pseudomonas* Species,

and *Salmonella* Species; and fungal strains like *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Fusarium solani*, *Alternata alternaria*, and *Schizophyllum* species were obtained from the Department of Microbiology, Government College University, Faisalabad-Pakistan. Well diffusion method was used for antibacterial and antifungal activities as described by Imran et al.,[16] Then Minimum Inhibitory Concentrations (MIC) of plant extract against two-gram positive bacterial strains including *S. aureus* and *B subtilis* and two-gram negative bacterial strains *E. coli* and *Acinetobacter* species were also investigated using microwell plate (96 wells) method. Streptomycin/ciprofloxacin as positive controls for bacteria and terbenafin for fungi were used. To evaluate the capacity of selected medicinal plant extract to prevent the biofilm formation against *S. aureus* and *B. subtilis* (gram positive bacteria); *E. coli* and *P. multocida* (gram negative bacteria) were also determined following the method of Di Ciccio et al. Finally, the Biofilm reduction (%) was calculated using the following formula

$$\text{Biofilm reduction}\,\% \;=\frac{(\text{OD control}-\text{OD sample})}{\text{OD control}}\times 100$$

In vitro Spermatozoa Parameters. Healthy volunteers (time-to pregnancy (TTP) 12 month) (n = 30) were chosen for the semen samples collection after taking written informed consent and semen samples were processed according to World Health Organization (WHO) protocol.[18] Different concentrations of extract (25 µg/mL, 50 µg/mL, and 100 µg/mL) were used to determine the impact on sperms motility total (%), progressive motility (%) and viability (%). After mixing the extract with semen (1:1), leave at room temperature for 30 seconds, then sperm motility total (%) as well as progressive motility (%) at specific times as at 0min, 15min, 30min, 45min, 60min, and 120min were recorded. .1% Eosin Y stain in .9% physiological saline was used to observe the viability of spermatozoa by incubating the semen, extract, and stain (1:1:1) for up to 120min following the protocol of Munir et al.[12]

Statistical Analysis

The obtained results were expressed as Mean ± SEM and further interpreted through statistical analysis by applying one way ANOVA test. Probability test was also used by Minitab 17 statistical software (Trial version).

Results

Results revealed the presence of steroids, flavonoids, tannins, saponins, and triterpenoids in the extract of *C. monnieri* while alkaloids and glycosides were not detected (Table 1). Significant ($p<.05$) amount of total phenolic contents and total flavonoids contents (309.33 ± 5.67 mg GAE/g and 68.64 ± 4.45 µg CE/g, respectively) in the extract of *C. monnieri* seeds were found (Table 2). HPLC-UV chromatogram of *C. monnieri* seeds extract' results revealed the presence of Gallic Acid (Rt = 2.830), Catechin (Rt = 3.097), P-coumeric acid (Rt = 5.499), HB acid (Rt = 7.061), and Ferulic acid (13.099) (Figure 1). The results of Fourier transform infrared spectroscopy (FTIR) (Figure 2) explored wide range of absorption peaks which represented the presence of wide range of bioactive natural chemical constituents with different functional groups like O-H, N-H, C-H, C≡C, C≡N, C=C, C=O, C-C, C-N, C-O, C-Cl, C-I, S-S, and N = O*.[12] The results of mineral contents, both essential and toxic elements, revealed significant ($p<.05$) concentration (ppm) in the seeds of selected medicinal herb (Table 3).

Table 1. Qualitative phytochemical constituents present in hydroethanolic extract of the studied medicinal plant.

Plant/Phytochemicals	*Cnidium monnieri*
Alkaloids	–
Flavonoids	+
Tannins	+
Saponins	++
Glycosides	–
Steroids	–
Triterpenoids	+

(+) indicates the detection of phytoconstituent, (–) indicates non detection of phytoconstituent present.

Significant ($p<.05$) hemolysis and clot dissolving activities as compared to controls (PBS) (Figures 3C and 3D) could be suggested that selected medicinal plant has significantly ($p<.05$) higher concentration of phytochemical constituents which might induce apoptosis in cells by destabilizing the cellular membrane.[19] The results of mutagenicity test explored that selected medicinal plant did not possess mutagenic activity and In vitro study using Fenton reaction results revealed comparatively with controls noticeable DNA damaging prevention potential of *C. monnieri* (Table 4 and Figure 4).

The results of antibacterial activity of selected natural herb evaluated by gel diffusion method are given in Table 5, reported as growth inhibition zones (mm) measured using a zone reader after incubating at 37°C for 24 hours. Results explored that selected medicinal plant possesses significant ($p<.05$) antibacterial activities against different selected pathogenic bacteria. It was found that *C. monnieri* showed at different extent antibacterial activities while did not show antibacterial activity against *P. multocida* and *K. pneumaniae* (Table 5). The results of MIC tested against *S. aureus*, *B. subtilis*, *E. coli*, and *Acinetobacter* species revealed that *C. monnieri* extract has highest growth inhibition potential against *S. aureus* and *Acinetobacter species* even in selected maximum dilution (.39 mg/mL). On the other hand, *C. monnieri* was only able to inhibit the growth of *B. subtilis* and *E. coli* upto concentrations of 12.5 mg/mL observed visually by change in color after adding 10 µL resazurin solution and the results were interpreted using the interpretation chart of NCCLS (1997). Furthermore, In vitro

Table 2. Phytochemical Constituents and antioxidant activities of selected plant hydroethanolic extract as mean ± SEM of multiple determinations of each experiment.

Plants\Contents	*C. monnieri*	Vitamin C
TPC (mg GAE/g dry plants material)	309.33 ± 5.67	—
TFC (μg CE/g dry plants material)	68.64 ± 4.45	—
H_2O_2 Scavenging activity (%)	17.67 ± 1.67	48.70 ± 2.91^{A}
DPPH Inhibition (%)	36.79 ± 1.78	90.15 ± 5.93^{A}

Mean with different letters as superscript in the same row indicate significant ($p<.05$) differences among tested plants extract and controls. Symbol – indicates not tested.

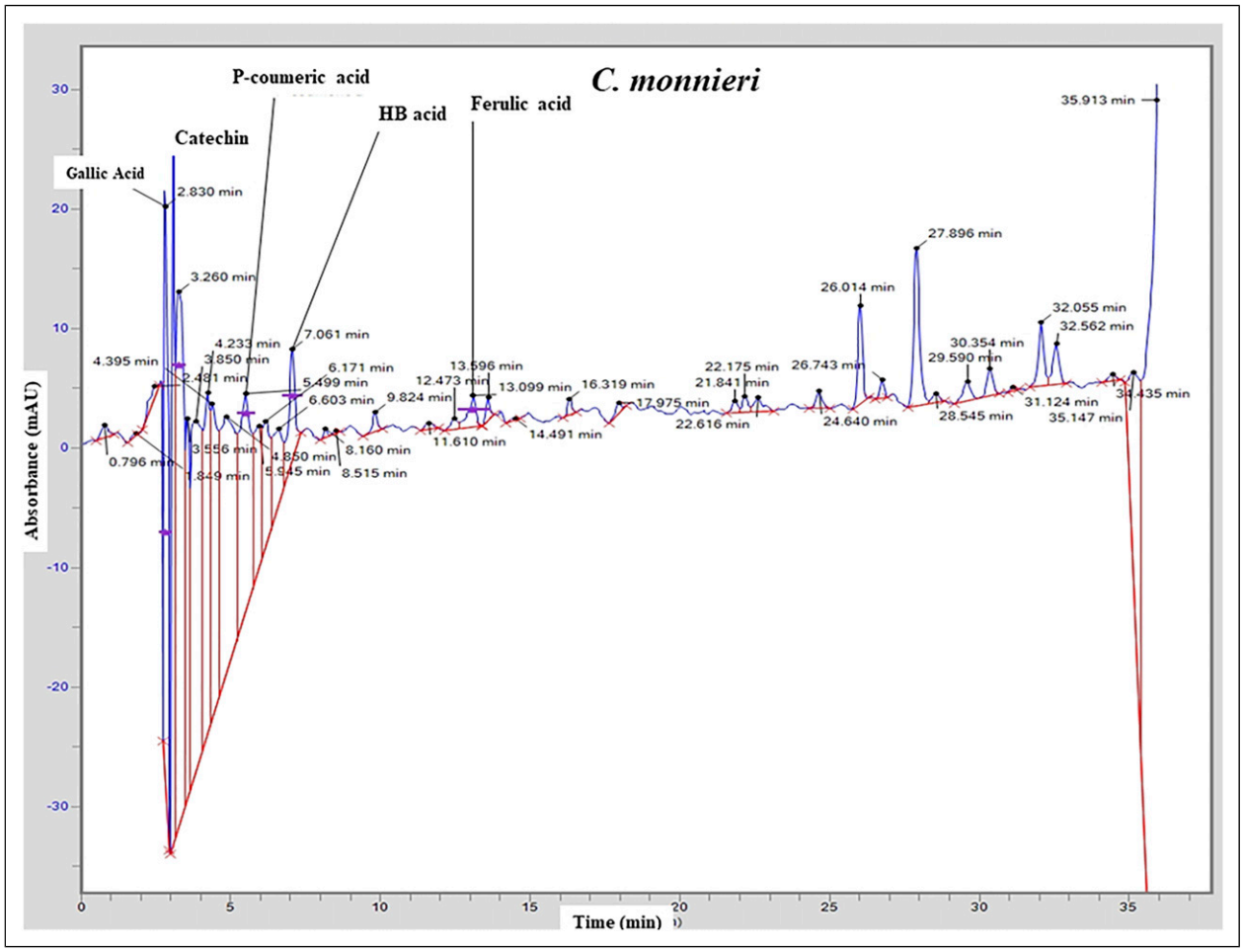

Figure 1. Chromatogram representing different phytochemical constituents identified using HPLC in the hydroethanolic extract of *Cnidium monnieri*.

potential of the selected medicinal plant to prevent the attachment and inhibition of biofilm formation was evaluated and results are given in Table 6. The results were calculated as Percent biofilm inhibition. *C. monnieri* seeds hydroethanolic extract have significant ($p<.05$) potential to prevent the attachment of *S. aureus, B. subtilis* and *E. coli* while enhanced the biofilm growth of *P. multocida* bacteria on the microwells plate (Table 6). The results of antifungal assay explored that *C. monnieri* has potential to inhibit the growth of *Schizophyllum* species, *A. flavus*, and *A. niger* at some extent but no activity against *F. solani,* and *A. alternaria* was observed (Table 7).

Results revealed that *C. monnieri* have significant ($p<.05$) spermicidal activity, observed on incubating the semen with hydroethanolic extract of *C. monnieri* on comparing with normal saline as control (Figure 5). Significantly ($p<.0$) decreased in motility both total and progressive of spermatozoa was observed on treating with hydroethanolic extracts of *C. monnieri* seeds. Moreover, it was also found that on treating

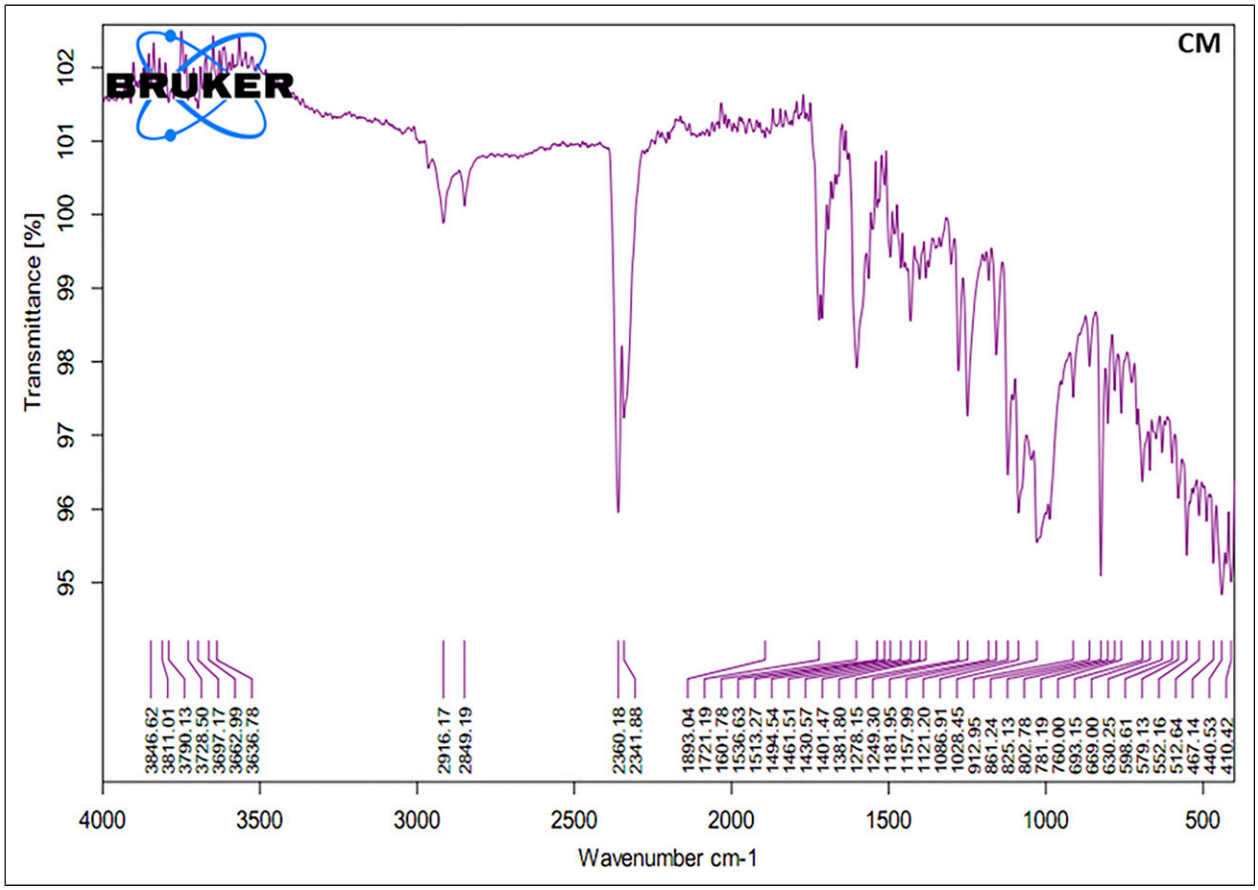

Figure 2. FTIR graph representing different functional groups and possible phytochemical constituents identified using FTIR in the hydroethanolic extract of *Cnidium monnieri*.

Table 3. Mineral contents of selected medicinal plant as mean ± SEM of multiple determinations of each experiment.

Plants\Contents	*C. monnieri*
Copper (ppm)	55.268 ± 2.17
Iron (ppm)	591.249 ± 8.80
Zinc (ppm)	36.856 ± 2.92
Magnesium (ppm)	215.817 ± 2.43
Manganese (ppm)	95.111 ± 2.91
Cobalt (ppm)	320.542 ± 5.68
Nickle (ppm)	71.778 ± 1.26
Cadmium (ppm)	4.572 ± .22
Lead (ppm)	2.633 ± 1.68

the spermatozoa with different concentrations of extract the viability of sperms significantly ($p<.05$) decreased, observed by increasing ability in retaining the eosin stain into the head of sperms (Figure 5A1-A3).

Discussion

The presence of a wide range of natural phytochemical constituents in the medicinal plants with multiple activities made them as a primary medicinal choice to compete many health-related issues among communities and individuals. It was well reported that the therapeutic applications like analgesic, antibacterial, and antispasmodic were due to the secondary metabolites derived from medicinal plants[20] and the significant antioxidant potential is due to the presence of phenolics contents.[21] Different phytochemical constituents isolated from *C. monnieri* mainly osthole and coumarins are responsible for the pharmacological activities of this medicinal plant.[22] Bio elements are classified in different groups as Group I to Group V based on their requirements and applications.[23] Figures 3A and 3B represented the potential antioxidant behavior of selected natural herb to neutralize the impact of free radicals. It was reported that long-term treatment of mitochondrial redox components with the herbal formulation containing Chinese herbal formula with extract of *C. monnieri* have diverse potential to enhance the antioxidant enzymes activities including GSH, α-tocopherol (α-TOC), and manganese-superoxide dismutase (Mn SOD).[24]

Evaluation of the herbs for genotoxicity assays is becoming very important worldwide to explore the possible potential hazards, and due to the reason, that only pharmaceutical compounds are been subjected for mutagenic activities.

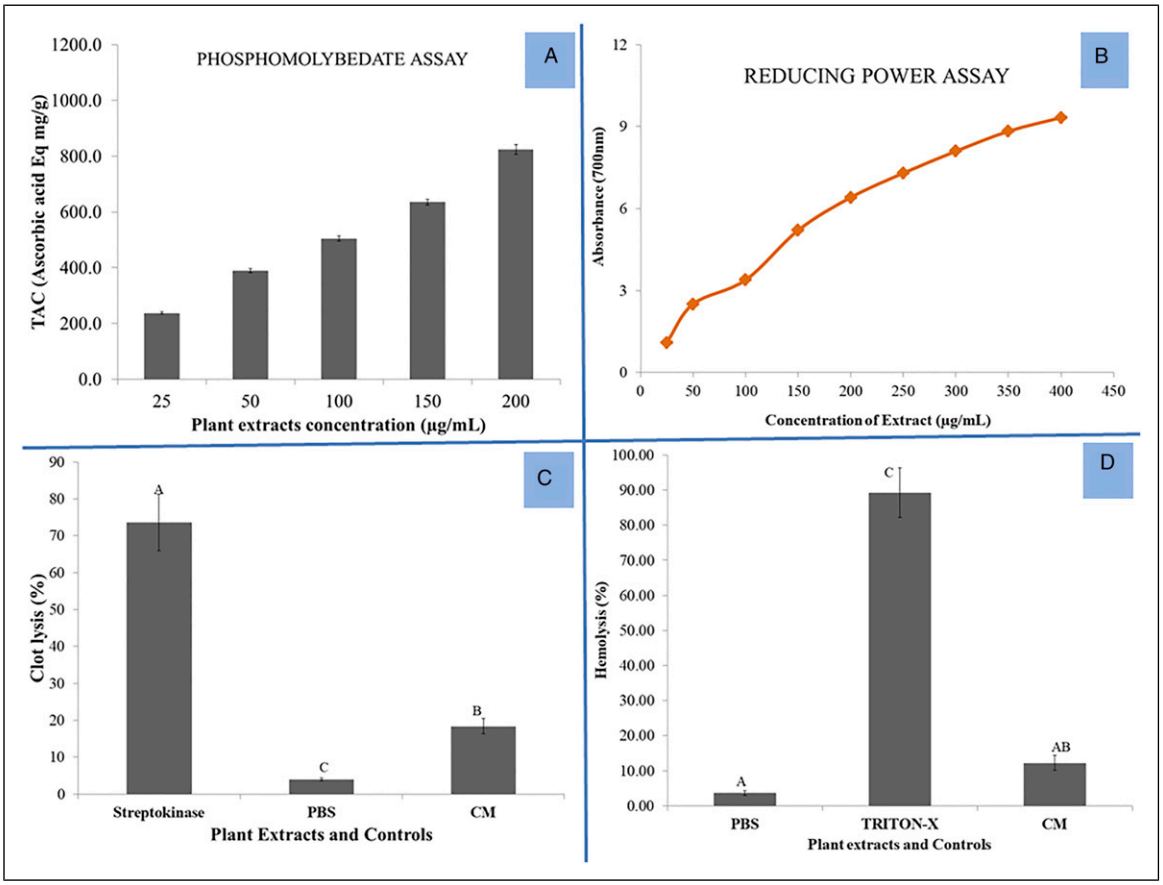

Figure 3. Represent the different biological activities of *Cnidium monnieri* (A) Total antioxidant capacity (TAC) by Phosphomolybdenum method, (B) reducing potential investigated by FRAP method, (C) hemolytic activities (%) evaluated against washed RBCs, and (D) thrombolytic activities (%) of selected medicinal plant. Where CM = *Cnidium monnieri*. The results are means ± SE of mean values of control and plant extract. Alphabets on the bars represent significance ($p<.05$) in group mean differences among tested plant extract and controls.

Table 4. Mutagenic activity of selected medicinal plant against S. typhi TA98 and TA100.

Hydroethanolic Extract and Bacterial Strains	Number of positive wells/ total number of wells	Results
Mutagenic activity against TA98	20/96	–
(a) Background		
(b) Standard (K2Cr2O7)	80/96	+
(c) C. monnieri	7/96	–
Mutagenic activity against TA100	25/96	–
(a) Background		
(b) Standard (NaN3)	86/96	+
(c) C. monnieri	5/96	–

+, Significant increase in the number of positive wells compared to the related control ($p < .05$). –, Non-significant ($p > .05$) effect observed.

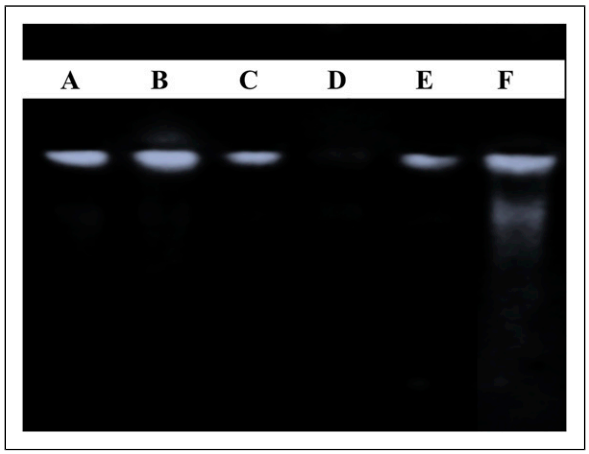

Figure 4. DNA damage prevention potential of selected medicinal plant using Ct DNA (Calf thymus DNA). Where, Lane A = Untreated DNA, Lane B = 2 mM $FeSO_4$, 30% H_2O_2 + DNA + 1 mM Quercetin, Lane C = 30% H_2O_2 + DNA, Lane D = 2 mM $FeSO_4$, 30% H_2O_2 + DNA, Lane E = 2 mM $FeSO_4$ + DNA, and Lane F = 2 mM $FeSO_4$, 30% H_2O_2 + sDNA + *Cnidium monnieri*.

Medicinal plants having genotoxic activities shall be announced genetically toxic and marked as unsafe.[25] Moreover, the evaluation of anti-mutagenic activities of medicinal properties is also very important to declare chemo-preventive

Table 5. Antibacterial activity of tested medicinal plant extract against selected bacterial strains.

	Antibacterial activity (Inhibition zones in mm)		
Bacterial Strains	*Cnidiummonnieri*	Streptomycin (1 mg/mL)	Ciprofloxacin (1 mg/mL)
S. aureus	08 ± .80	36 ± 2.70	
B. subtilis	13 ± 0.9	35 ± 2.10	
E. coli	17 ± 1.90		34 ± 2.00
P. multocida	–		32 ± 1.90
Acinetobacter species	13 ± .21		37 ± 2.10
Pseudomonas species	11 ± .44		33 ± 1.54
Salmonella species	06 ± .33		35 ± 1.71
K. pneumoniae	–		28 ± 1.39

Values are Mean ± SE (standard error mean) of replicate measurements. Values with different alphabets in superscripts within same rows are significantly different ($p<.05$), Note: (–): no activity observed, mm (millimeter inhibition zone).

Table 6. Biofilm formation inhibition Assay to evaluate the biofilm inhibition potential of selected medicinal plant extract against selected bacterial strains.

		Biofilm formation inhibition (%)			
Plants Extracts/Bacterial Strains		*S. aureus*	*B. subtilis*	*E. coli*	*P. multocida*
C. monnieri	10 mg/Ml	$48.02 \pm .65^{C}$	85.31 ± 1.37^{B}	67.55 ± 1.44^{B}	-1.07 ± 1.08^{B}
	20 mg/mL	65.75 ± 1.73^{B}	85.82 ± 1.91^{B}	77.83 ± 1.24^{B}	-6.61 ± 1.04^{B}
Streptomycin (1 mg/mL)		85.69 ± 1.44^{A}	94.32 ± 1.34^{A}	80.30 ± 1.67^{A}	51.82 ± 2.00^{B}
Ciprofloxacin (1 mg/mL)		$77.64 \pm .99^{A}$	91.90 ± 2.46^{A}	76.00 ± 1.22^{A}	59.35 ± 1.24^{A}

Values are Mean ± SE (standard error mean) of replicate measurements. Values with different alphabets in superscripts within same column for same concentrations are significantly different ($p<.05$), Note: − sign indicate the enhancement of biofilm formation. The numbers > 0% ≥ 50% show low activity, >50% (in bold) show high activity against the bacteria.

Table 7. Antifungal activities of selected medicinal plant aqueous ethanolic extract against selected fungal strains.

	Antifungal activity (Inhibition zones in mm)	
Bacterial Strains	*Cnidiummonnieri*	Terbenafin (1 mg/mL)
Schizophyllum species	5 ± .36	22 ± 1.91
F. solani	–	11 ± 1.1
A. alternaria	–	26 ± 2.2
A. flavus	8 ± .38	29 ± 1.3
A. niger	7 ± .42	28 ± 2.0
A. terreus	6 ± .29	31 ± 1.99

Values are Mean ± SE (standard error mean) of replicate measurements. Values with different alphabets in superscripts within same rows are significantly different ($p<.05$), Note: (–): no activity observed, mm (millimeter inhibition zone).

characteristic of medicinal plants and therapeutic potential for clinical purpose use. Anti-mutagens compound prevents the mutagenicity might by neutralizing the mutagen or preventing the reaction of DNA and mutagens.[26] The concern related to the safety of medicinal plants is becoming one of the emerging interests for researchers and the investigation of toxic aspects of herbs that might be associated with their usage, therefore avoiding potential harmful effects.[27] The results of mutagenicity test explored that selected medicinal plant did not possess mutagenic activity or in other words selected natural herb is non-mutagenic in nature. According to literature review, this was the first time that selected medicinal plant was screened out for the mutagenic activities.

Furthermore, medicinal plants having significant ($p<.05$) antibacterial potential could be used as medication for the treatment as well as prevention of infectious diseases.[28] Biofilm formation is one of the major resistance mechanisms against available antibiotics of different bacterial strains which produced the resistance maintenance capability in microbes, modulate the ability of transmission, and increased the reversibility potential.[29,30] Our results agreed with the findings of Alam et al.,[31] who reported that methanolic extract of *Bacopa monnieri* L. has significant antimicrobial activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *K. pneumonia*, and activities against *S. aureus* and *E. coli* were also reported.[32] Alam et al.,[31] investigated that *B. monnieri* L. have antifungal activities to inhibit the fungi like *C. albicans* (UCC 29), *Microsporum audouinii* (MUCC 545), *A. niger* (MUCC 177), and *Trichophyton mentagrophytes* (MUCC 665) when extracts are used in different concentrations as using the method of Kirby–Bauer disk diffusion. *B. monnieri* also have potential

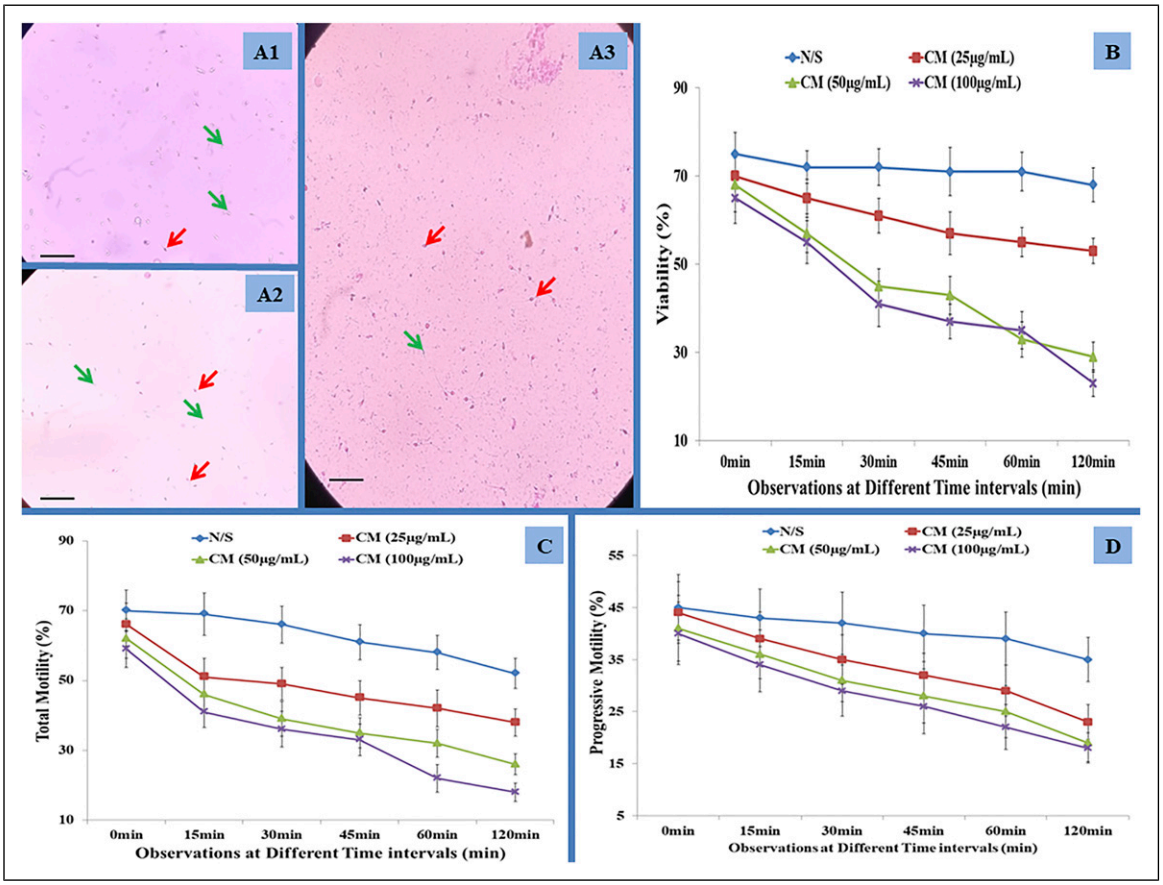

Figure 5. In vitro spermicidal activity evaluated using human sperms (A1) Eosin stained sperms treated with normal saline at 0 time interval (A2) Eosin stained sperms treated with hydroethanolic extract at 0 time interval (A3) Eosin stained sperms treated with hydroethanolic extract at 120 min time interval (B) Viability (%) of Spermatozoa (C) Total Motility (%) of Spermatozoa (D) Progressive Motility (%) of Spermatozoa evaluated at different time intervals (minutes) after incubating with the extract of selected medicinal plant extract and control. The values are mean ± SEM of replicate determinations. Where N/S = normal saline, CM = *Cnidium monnieri*, Green arrow represent the viable sperm (white headed sperm), Red arrow represent the nonviable sperms (pink colored headed sperms), black line scale bar (50 µm).

against *A. flavus*, and *C. albicans*.[32] Experiments revealed that osthole inhibits *Fusarium graminearum*, *Aparagillus* species habitats on common weeds and cereal crop. *Sphaerotheca Fuliginea* regulated by spore-spreading and mycelium growth inhibitions was also reported.[33]

Therapeutic as well as nutraceuticals properties of medicinal plants are being accepted worldwide particularly in developing countries for the management of different disorders. The findings of spermicidal studies explored significant ($p<.05$) contraceptive pharmacological behavior of *C. monnieri.* Our results agreed with the finding of Yingzi[34] and Shuying.[35] Yingzi[34] explored the spermicidal mechanisms of traditional Chinese medicine *C. monnieri,* by observing the ultrastructural changes in human sperm with this natural herb. The sperm membrane in both head and tail regions were damaged significantly, the acrosomal membrane and nuclear membrane were disrupted, the mitochondria were injured, and vacuolization were seen in it; the part microtubule were injured and dissolved. On the other hand, Shuying,[35] observed the spermicidal effect of *C. monnieri* at different times intervals on semen samples obtained from 18 healthy men and found that *C. monnieri* has a definite spermicidal effect on spermatozoa in vitro. Our results also revealed a close relationship between the spermicidal effect, the concentration and dosage of *C. monnieri* extract. *C. monnieri* may be a new kind of spermicidal contraceptive per vagina. But In vivo study in animals and clinical trials are required to declare this natural herb as therapeutic agent.

Conclusion

Since earlier civilizations, natural medicinal plants have been used by human being for their survival and growth. Over the years, therapeutic as well as nutraceuticals properties are being accepted worldwide particularly in developing countries for the management of different disorders. A wide range of bioactive molecules in the hydroethanolic extract of *C. monnieri* having various biological activities have been

investigated. Moreover, In vivo cytotoxic studies revealed that this medicinal herb is non-toxic and non-mutagenic in nature having significant antimicrobial activities. Furthermore, spermicidal response against human spermatozoa revealed contraceptive per vaginal behavior of this natural medicinal plant. Although, *C. monnieri* showed pharmacological activities with non-toxic behavior but In vivo study in animals and clinical trials are required to declare this natural herb as therapeutic agent.

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