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Protective Evidences of *Trachyspermumammi*MethanolicSeed Extract on *E. coli*Induced Peritonitis in *Albino wistar*Rats and its Antioxidant Activities

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Abstract:

Peritonitis is an infection caused by bacteria in the normal germ free area of the peritoneal cavity.*Trachyspermumammi*isan ayurvedic formulation proven to be effective in the therapy of abdominal infection.The objective of the present study was evaluate the antimicrobial and protective potential of the methanolic of*Trachyspermumammi* in *E. coli* induced model of peritonitis in albino wistar Rats. Rats were pre-treated with 200 mg/kg and 400 mg/kg/bwt dose for 3 days and fourth day with *E. coli* (1×10^8 CFU/ml) strain and consecutively 3 days treatment. Mortility was monitored for 14 days. After the death of rats or completion of the experiment rats were sacrifice and kidney were used for our protocol.Colonies were count and statically analysis was done. Results showed dose dependent anti-microbial activity. Thus the methanolic extract of *Trachyspermumammi*exhibited significant protection against *E. coli* induced peritonitis in normal rats.It significantly reduced the viable cells of *E. coli* when inoculated in rats. Activity is attributed to flavonoids and tannins. The present study thus suggests that Methanolic extract of *Trachyspermumammi* significantly reverses peritoneal infection by *E. coli* in rats. It can be suggest that this medicinal formulation will be used as herbal medicine with no side effects.

Keywords: Peritonitis, *Trachyspermumammi*,MIC,*E. coli* strain, Ajwain

Introduction:

There has been a rising interest in antimicrobial herbal drugs in the last years which may be explained by the occurrence of multiresistant pathogens and by increasing popularity of alternative medicine. Indeed, plants use a huge, mainly unknown reservoir of substances for their defense against microorganisms, insects, and herbivors. Although some single substances like phenols, phenolic derivatives (quinones, flavones, flavonoids, flavonoles, tannins, and cumarins), terpenoids, essential oils, alkaloids, lectins, and polypeptides have been identified (Cowan, 1999), whole extracts of plants are still in use. Peritonitis is a common and frequently fatal bacterial infection of ascites occurring in patients with cirrhosis who have diverse symptomatology. The diagnosis is distinct from secondary peritonitis and hence is made in the absence of an intra-abdominal source of infection or inflammatory process. SBP was first described in 1907 by Krencker followed by Caroli in 1958 and Kerr and colleagues in 1963. (Krencker*et al.*,1907, Caroli*et al.,* 1958 and Kerr *et al.*,1963)

A variety of factors are associated with the development of Peritonitis including the patho-physiological hallmark: bacterial translocation in an immune-compromised host. The incidence of Peritonitis ranges from 10% to 30%13 and mortality from 10% to 46%. (Rimola*et al.,* 2000 and Guarner*et al.*,1995).In a healthy individual, the variety and density of bacteria increases exponentially from the stomach to the colon with up to a 1000 or more different species and a trillion bacteria per gram of faecal material in thecaecum.(Marchesi*et al.,* 2007) A symbiotic relationship usually exists. However, in advanced liver

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disease, normal intestinal flora can cause deleterious effects to the host through a variety of mechanisms leading to SBP including bacterial overgrowth (Guarner*et al.*, 1997 and Bauer *et al.*, 2001). Known as Ajwain, *Trachyspermumammi*(L.) Sprague is an annual herbaceous plant belonging to the highly valued medicinally important family, Apiaceae (Gersbach*et al.*, 2002) It is said that the herb is widely grown in arid and semi-arid regions where the soil involve high amount of salts (Joshi *et al.*, 2000). The herb is generally grown in October–November and should be harvested in May–June (Chauhan *et al.*, 2012 and Ranjan*et al.*, 2012). Oral application of seed was reported to be useful for paralysis, tremor and palsy as well as other neural disorders in the field of neurology (Aghili*et al.*, 1992) Fruits were widely administered for liver spleen as well as gastrointestinal disorders such as nausea, vomiting, reflux, abdominal cramps and loss of appetite (Aghili*et al.*, 1992), beneficial in stomach troubles and possess stimulant and carminative properties (Tonekaboni*et al.*,2007) anthelmintic medicine and also antidote for various natural toxic agents (Tonekaboni*et al.*,2007). Accordingly, both total alcoholic extract and total aqueous extract possess *in vivo* signi-ficant anti–inflammatory effect (Thangam*et al.*,2003).

Material and method:

Collection and authentication of plant sample:-

The plant *Trachyspermumammi* (seeds) were collected from the local grocery shop New market Bhopal city (M.P.).The taxonomic identification of plant confirmed by Dr. Zia-Ul -Hassan, Head, Department of Botany, Govt. Sefia Science College ,of Science ,peer get, Bhopal India. The herbarium was submitted to the department of botany, Govt. Safia College of Science, peer get, Bhopal, M. P. India and the voucher no. 181/Bot/Saf/17

Bacterial culture

The test organisms *Escherichia coli* (*MTCC* 2075) were obtained from the stocks of the Pinnacle Biomedical Research Institute, Bharat scout guide bhawan, shyamla hills, Bhopal, (M.P.)

Preparation and Extraction of plant Material

Dried plant material was coarsely milled. The extraction done on crude powdered is placed in a stoppered container with the solvent and allowed to stand at room temperature for a least 2 days for petroleum ether and 5 days for methanol with frequent agitation until the soluble matter has dissolved. The mixture then is strained, the mare were seprated out, and the combined liquids are clarified by filtration or decantation after standing (Sangeetha J. *et al.*, 2011).

Phytochemical investigation:

Quantitave phytochemical investigation: The phytochemical investigation was carried out by procedure given byKokate*et al.*, 2006.

Total Phenolic Content Estimation

The folin-Ciocalteu reagent is sensitive to reducing compounds including polyphenols.They produce a blue colour upon reaction.This blue colour was measured spectro-photometrically.Put absorbance of test sample in line of regression of standard curve of gallic acid.Calculate total phenolic content. Express as mg/gm or, µg/mg galic acid equivalent. (Ainsworth *et al.*, 2007)

Total Flavonoid Content Estimation

Flavonoid contents were carried out by reaction of $NaNO_2$ and $AlCl_3$ by preparing different concentration of Rutin in methanol.Take absorbance of the mixture at 510 nm versus a prepared water blank.Put

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absorbance of test sample in line of regression of standard curve of Rutin. Calculate total flavonoid content. Express as mg/gm or, µg/mg Rutinequivalent(Zhishen*et al.*, 1999)

Antioxidant activity:

DPPH radical scavenging activity

Prepare different concentration of test sample with methanol. Add 2ml of test sample and 1ml of DPPH (1mM) solution. Incubated it for 30 min at room temperature. Take absorbance at 515 nm against blank (methanol). Calculate % Inhibition = [(AC 515 nm- AS 515 nm/ AC 515 nm) x 100]. Plot a curve for % Inhibition and concentration and using line of regression estimate IC50.(R Jain 2011).

Superoxide scavenging assay:

This assay were carried out by the reaction of NBT and Alkaline DMSO by preparing different concentration of test sample.The absorbance will be measured at 560 nm. Plain DMSO used as blank and reaction mixture without extract (water in place of extract) used as control. Decreased absorbance of the reaction mixture indicated the increasing of superoxide anion scavenging activity. 50% inhibition of extract was calculated by plot a graph between absorbance and concentration.(Veerapur*et al.*, 2009)

Anti bacterial sensitivity assay

Well diffusion method

The agar well diffusion method technique (Bauer *et al.*, 1966) was used to determine the antibacterial activity of the plant extracts. Inoculation was done on sterile nutrient agar media plate using 18 hours old culture. A sterile 5mm cork borer was used to punch holes after solidification of media. The wells formed were filled with different concentrations of the extract which were labeled accordingly; 100mg/ml, 150mg/ml, 200mg/ml, 250mg/ml. The plates were then left on the bench for 1 hour for adequate diffusion of the extracts and incubated at 37°C for 48 hours in upright condition. The Experiment was repeated triplets and the mean values were calculated.

Minimum inhibitory concentration (MIC) Assay:

The MIC is the lowest concentration of a substance that inhibits the growth of fungi within a defined period of time. The MIC is expressed in mg/ml. Broth dilution is a susceptibility testing technique in which serial dilutions (usually two-fold) of an antifungal agent are made in a liquid medium that is inoculated with a standardised number of organisms and incubated for a prescribed period. The objective of this method is the determination of the MIC. (Rodriguez *et al.*, 2008)

***In vivo* study**

Animals:

The four week old *Albino wistar* Rats (200±30gm) with a background were obtained from Pinnacle Biomedical Research Institute, Bhopal (M. P). All animal experiments were conducted and approved by the Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal (CPCSEA Reg. No. 1824/PO/ERe/S/15/CPCSEA, Protocol Approval No. PBRI/IAEC/PN-417025).

Acute oral toxicity (OECD 423, 2001)

The acute toxic class method set out in this Guideline is a stepwise procedure with the use of 3 animals of a single sex per step. Depending on the mortality and/or the moribund status of the animals, on average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. The substance is administered orally to a group of experimental animals at one of the defined doses. The substance is tested using a stepwise procedure, each step using three animals of a single sex. Absence or presence of

compound-related mortality of the animals dosed at one step will determine the next step, i.e.; no further testing is needed, dosing of three additional animals, with the same dose and, dosing of three additional animals at the next higher or the next lower dose level. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels, 5, 50, 300 and 2000 mg/kg body weight.

Grouping of animal:

Group-I:*Escherichia coli*(1×10^8 CFU/ml) treated group

Group-II: 200 mg/kg extract treated group

Group-III: 400 mg/kg extract treated group

Group-IV: Standard drug (Ofloxacin-5m g/kg body weight) treated group

Preparation for bacterial inoculums

In brief, *E. coli* strain (MTCC 2075) were grown on pre-sterile Nutrient broth Medium from a single colony and incubated at 37°C for 16–18 h at 37°C to obtain stationary growth phase cultures. The bacteria were then centrifuged (200 rpm) for 10 min at 4°C, and the pellets were resuspended in PBS to an OD of 0.1 at 660 nm, with a spectrophotometer, corresponding to 10^8 CFU/ml.(Teixeira-da-Cunha *et al.,* 2013)

Systemic infection by *E. coli*:

To produce infection, the rats were induced by the intra-peritoneal with suitable inoculums in a volume of 0.2 to 0.25 ml. After infection, the rats were observed twice daily, and animals exhibiting profound inanition or an inability to reach food and water were sacrificed. The experimental design involved administration of each of the three test agents by daily oral dosing for a period of 7 days. dosing regimens were started on days -1,-2, -3, 0, 1, 2, 3 and relative to the day of challenge (day 0) with 2×10^4 CFU of *E. coli* CFU/ml. Before and after the challenged day animals were treated with 200 mg/kg body weight and 400 mg/kg body weight*Trachyspermumammi*seed extract and 5 mg/kg body weight Ofloxicin respectively. Survival was monitored for all experimental groups till 14 day.These conditions were in accordance with those of previously described method[Ikeda *et al.,* 2000], with slight modification. The pathological status of the rat was determined by visual examination of internal organs after their death or sacrifice at the completion of the experiment. All surviving rats were killed by cervical dislocation on 15 day determination of the numbers of CFU of *E. coli* per gram from the kidney [Donna *et al.,* 2004 and Rex *et al.,*1998]. This determination was made by aseptically removing and weighing both kidneys, homogenizing kidneys in w/v ml of saline with a High Speed Homogeniezer (Remi RQ-124A), and Kidney burden was determined by culturing of homogenates in physiological saline followed by plating 0.1 ml aliquots onto Nutrient agar plates. The plates were incubated at 37°C, and the number of colonies was enumerated after 48 h of growth [Arthington*et al.,* 2000]. All animal care procedures were supervised and approved by the Institutional Animals Ethics Committee (IAEC) of PBRI, Bhopal.

Monitoring of mortality:

In survival studies 6 rats per treatment group were inoculated with *E. coli*. Because mortality. Occurs primarily between 24 and 48 h after infection in this model, mortality was assessed every 2 h in this period; thereafter, mortality was monitored every 6 hours.

Histology:

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During the collection of the tissue from body of rat for the study of structural changes, pieces of tissue were cut washed and transferred in 10% formalin solution. After that various syaining were done. Then slide were examined under microscop. (Slaoui, M., &Fiette, L. 2011).

Biostatical interpretation

All data are presented in Mean ±SD. Data were analyzed by One Way ANOVA followed by Bonfeffoni's test. $P<0.05$ was considered as level of significance (n=4).

Results:

Table 1: Qualitative Photochemical investigation:

S. no.	Phytoconstituents	Test name	Methanol	Pet ether
1.	Saponins	Froth	Negative	Negative
2.	Carbohydrates	Molisch Benedict	Possitive	Possitive
3.	Protein & Amino acid	Biuret	Negative	Negative
4.	Glycosides	Borntrager	Negative	Negative
5.	Alkaloids	Mayer's Wagner's Hager's	Possitive	Possitive
6.	Flavonoid	Alkalin reagent Lead acetate	Possitive	Possitive
7.	TNS	Salkowski	Steroid Possitive	Negative
8.	TNP	Ferric chloride dilute iodine	Possitive	Negative

Quantitative Photochemical investigation:

Table 2: Total phenolic content of gallic acid

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.1098
2.	40	0.1763
3.	60	0.2468
4.	80	0.2981
5.	100	0.3258

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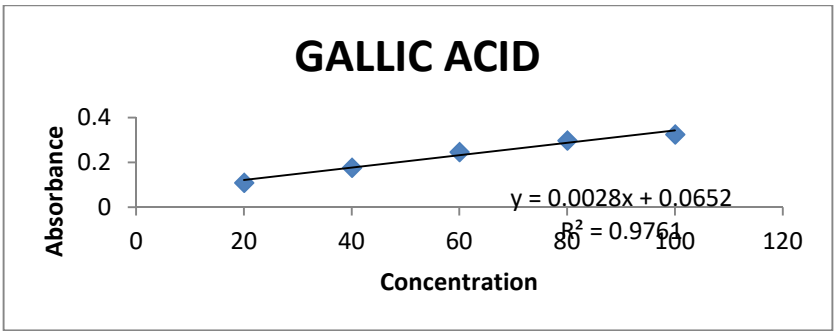


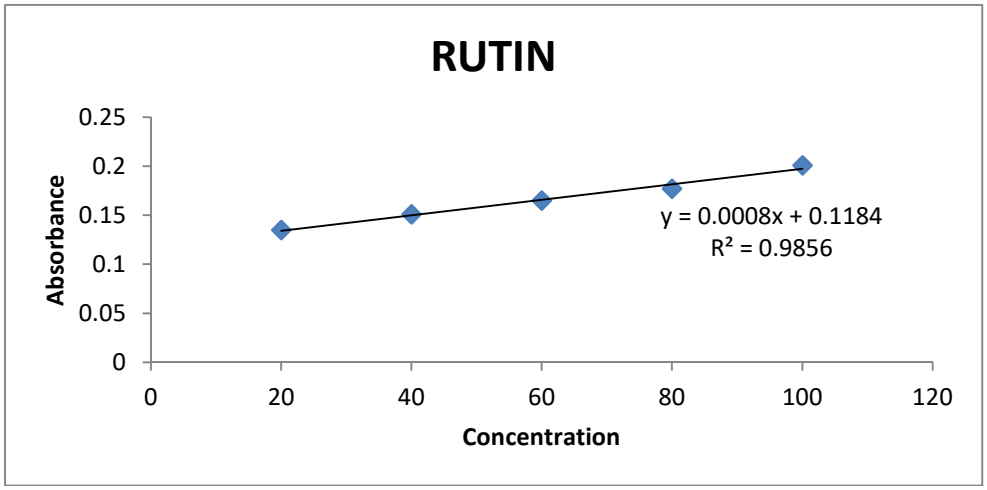
Graph 1: Total phenolic content of gallic acid

Table 3: Total phenolic content of Trachyspermumammimethanolic extract

S. no.	Concentration (µg/ml)	Absorbance
1.	100µg/ml	61.26±0.115

Table 4: Total flavanoid content of Rutin

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.135
2.	40	0.151
3.	60	0.165
4.	80	0.177
5.	100	0.201



Graph 2: Total Flavonoid content of rutin

Table 5: Total flavonoid content of extract

S. no.	Concentration (µg/ml)	Absorbance

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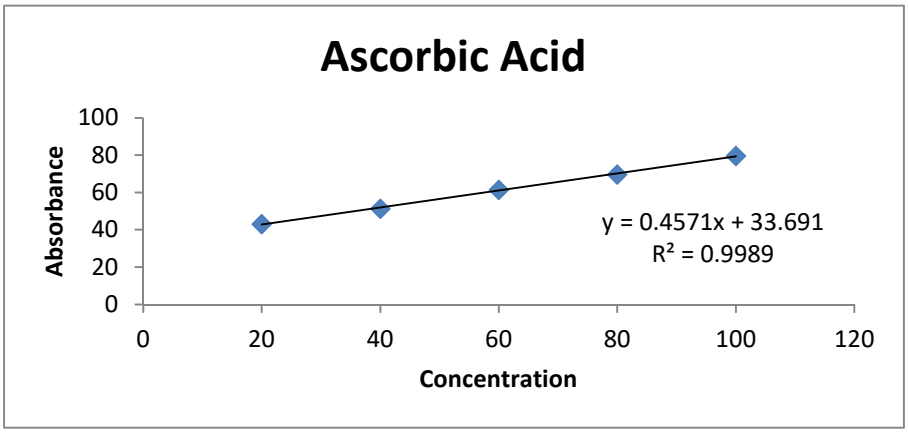
1.	100 µg/ml	72.33±0.577

Antioxidant activity

Table 6: DPPH of Ascorbic acid

S.No.	Concentration (µg/ml)	Absorbance
1.	20	0.397
2.	40	0.34
3.	60	0.269
4.	80	0.211
5.	100	0.142



Graph 3: DPPH of Ascorbic acid

Table 7: DPPH of Methanolic extract of Trachyspermumammi

S. No.	Concentration (µg/ml)	Absorbance
1.	20µg/ml	0.235
2.	40 µg/ml	0.202
3.	60 µg/ml	0.122
4.	80 µg/ml	0.099
5.	100 µg/ml	0.085

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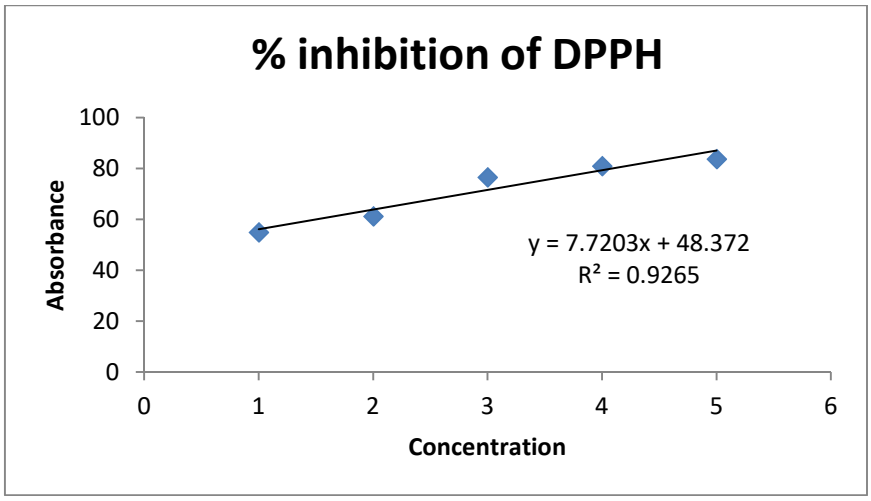
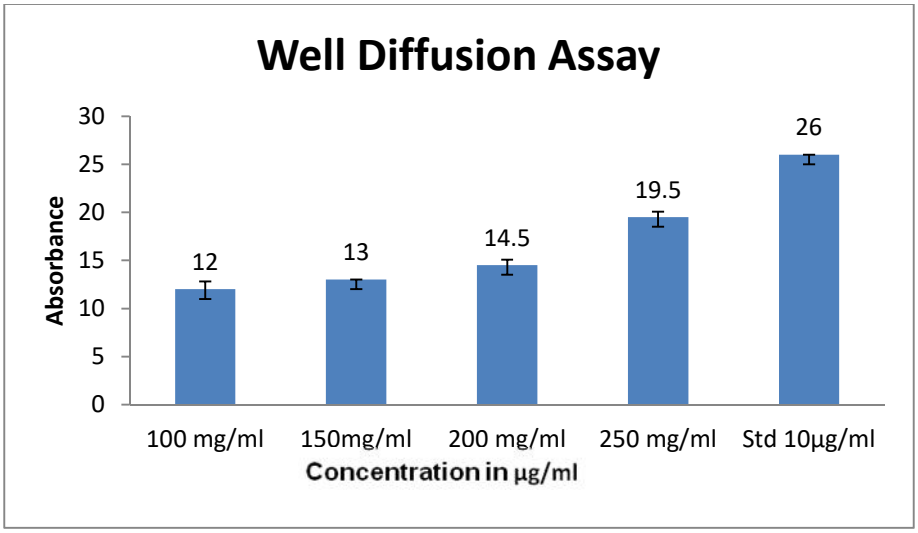


Graph 4: DPPH of Methanolic extract of *Trachyspermumammi*

Table 8: Well Diffusion Assay

S. no.	100mg/ml	150mg/ml	200mg/ml	250mg/ml	Standard (10µg/ml)
1.	12.00±0.816	13.00± 0.000	14.50± 0.577	19.50± 0.577	26.00± 0.000



Graph 5: Well Diffusion Assay of methanolic extract of *Trachyspermumammi*

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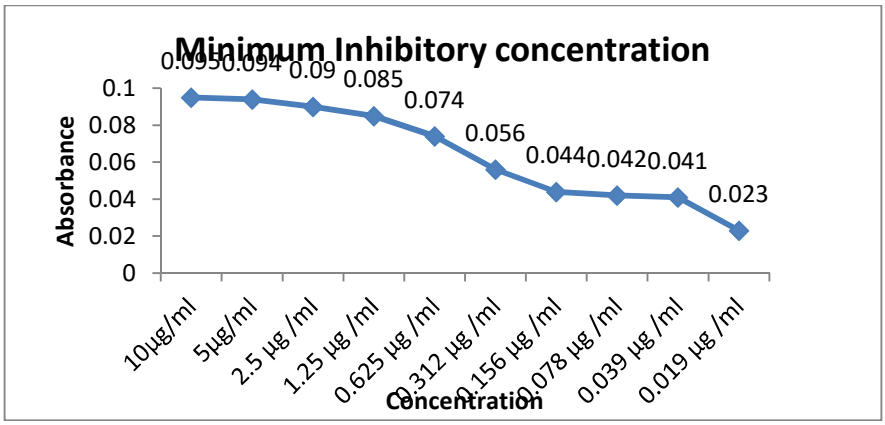
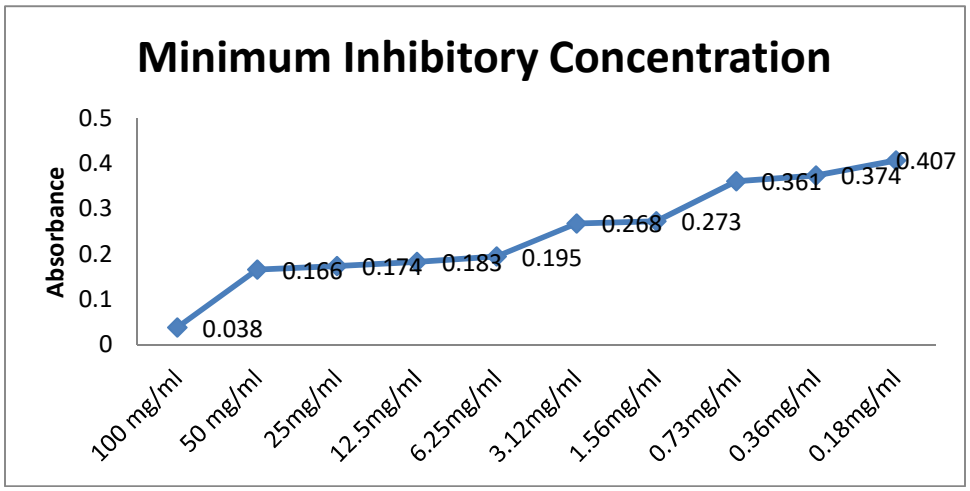


Graph 6:MIC of Ofloxicin



Graph 7:MIC of methanolic extract of *Trachyspermumammi*

Table 9:Acute oral toxicity

Group	Dose (mg/kgbw)	Weight (gm)	No. of rat (Tested/ Survived)
A	5 mg/kg	194	3/3
B	50 mg/kg	190	3/3
C	300 mg/kg	185	3/3
D	2000 mg/ml	182	3/3

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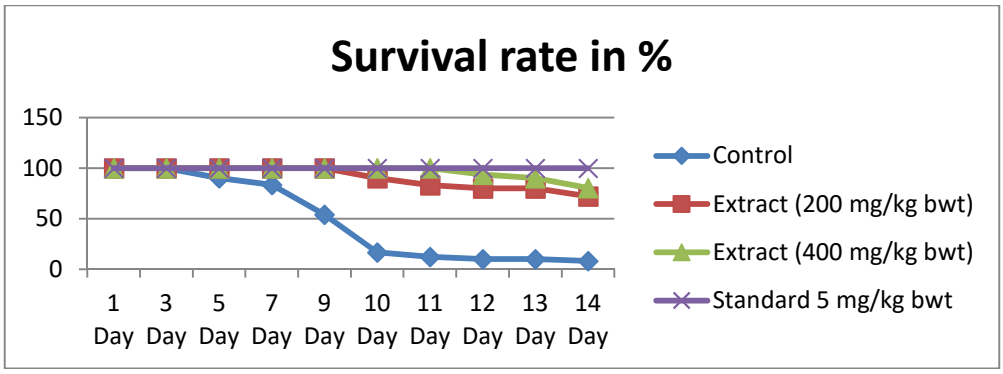
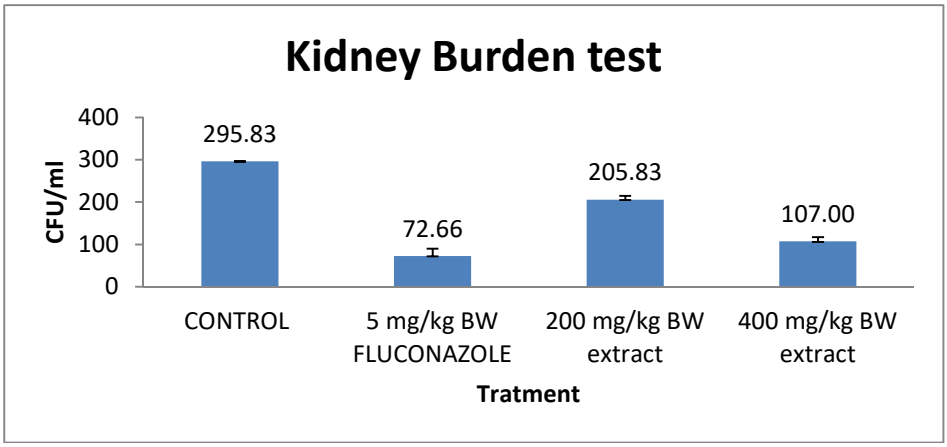


Graph 8: Survival rate in %

Table 10: Kidney burden test

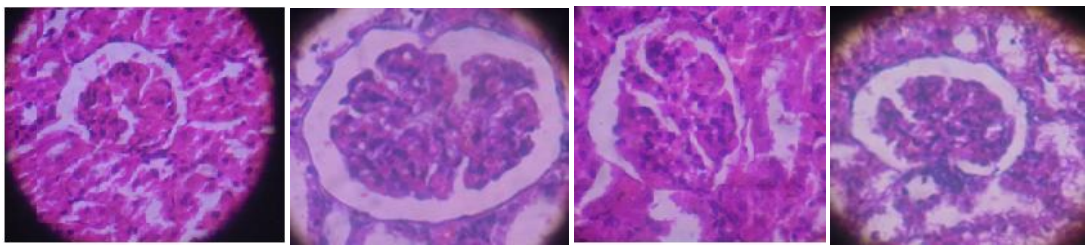
S. No.	Groups	CFU/ml
1.	Control *E. Coli* treated	295.83±1.169
2.	Standard drug (ofloxicin 5 mg/kg bwt)	72.66±17.603**
3.	Extract 200 mg/kg bwt	205.83±*8.472***
4.	Extract 400 mg/kg bwt	107.00±*10.139***

Our data were represent mean±SD by software STAT 32 .data were compared with control group ** is represent as p<0.001, *p<0.050, and non significant as (ns).



Graph 9: Kidney burden test

Histology of kidney:



G-I *E. coli* treated | **G-II Ofloxicin mg/kg bw** | **G- III Extract 200mg/kgbw** | **G-IV Extract 400 mg/kgbw**

Discussion:

Peritonitis is a severe infection with high mortality occurring in 7–31% of hospitalised patients with cirrhosis and ascites (Borzio*et al.,* 2001) and its prevalence, among other infections, is increasing in such patients across the United States.(Singal*et al.,* 2014)In Indian system of medicine, ajwain is administered for stomach disorders, a paste of crushed fruits is applied externally for relieving colic pains; a hot and dry fermentation of the fruits was lapped on the chest to cure asthma. Aqueous extract of ajwain which was also known as Ajwan-ka-arak used for the treatment of diarrhoea (Krishnamoorthy and Madalageri 1999; Soni*et al.,* 2016).The present investigation revealed that the *T. ammi* extract have some major bioactive components which helps to heal the peritoneal cavity from the infection. It has contents of flavonoid and phenols which is relatively to the standard drugs, like this it has antioxidant activity. The extract showed the zone of inhibition in *In vitro* antimicrobial activity. After all the experiment *in vivo* study were done and it shows relative significance to the standard drugs.Ajwain has been well known as an Ayurvedic spice since ancient times. It has traditionally been used as a medicinal plant for the treatment of indigestion and dyspepsia and many other gastric disorders. Ajwain is also rich in moisture, protein, carbohydrates, fat, minerals, fiber, calcium, phosphorus, iron, carotene, thiamine, riboflavin and niacin. Chemical composition of essential oil and presence of variety of diverse constituents in it are responsible for a wide range of biological properties. Our present investigation reveals the properties of *T. ammi* which hous hold medicine, it cures the peritoneal infection.

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Reference:

- Aghili-Shirazi S. MakhzanolAdvieh. 1st ed. Tehran: IntisharatvaAmoozeshenghelabIslami Press; 1992.
- Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissue using Folin-Ciocalteu reagent. Nature Protocol, 2007; 2(4): 875-877
- Arthington-Skaggs, B. A., Warnock, D. W., & Morrison, C. J. (2000). Quantitation of Candida albicansergosterol content improves the correlation between in vitro antifungal susceptibility test results and in vivo outcome after fluconazole treatment in a murine model of invasive candidiasis. Antimicrobial agents and chemotherapy, 44(8), 2081-2085.
- Bauer TM, Steinbruckner B, Brinkman F, et al. Small intestinal bacterial overgrowth in patients with cirrhosis: prevalence relation with spontaneous bacterial peritonitis. Am J Gastroenterol 2001; 96: 2962–7.
- Bauer, A. W., Kirby, W. M. M., Sherris, J. C., &Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 45(4), 493.
- Borzio M, Salerno F, Piantoni L, et al. Bacterial infection in patients with advanced cirrhosis: a multicentre prospective study. Dig Liver Dis 2001; 33: 41–8.
- Caroli J, Platteborse R. Portocavalsepticemia; liver cirrhosis &septicemia caused by colibacillus. Sem Hop 1958; 34: 472–87.
- Chauhan B, Kumar G, Ali M. A Review on phytochemical constituents and activities of *Trachyspermumammi*(L.) sprague fruits. AJPTR. 2012; 2(4):329–40.
- Cowan, M. M.: Plant products as antimicrobial agents. Clin. Microbiol. Rev. 12, 564 ± 582 (1999).

- Donna M. MacCallum and Frank C. Odds (2004) Need for Early Antifungal Treatment Confirmed in Experimental Disseminated Candida albicans Infection. Antimicrobial agents and chemotherapy, 48(12), 4911–4914.
- Gersbach PV, Reddy N. Non-invasive localization of thymol accumulation in *Carumcopticum*(Apiaceae) fruits by chemical shift selective magnetic resonance imaging. Ann Bot. 2002; 90(2):253–57.
- Guarner C, Runyon BA, Young S, et al. Intestinal bacterial overgrowth and bacterial translocation in cirrhotic rats with ascites. J Hepatol 1997; 26: 1372–8.
- Guarner C, Runyon BA. Spontaneous bacterial peritonitis: pathogenesis, diagnosis, and management. Gastroenterologist 1995; 3: 311–28.
- Ikeda. F., Y. Wakai, S. S. Matsumoto, K. Maki, E. Watabe, S. Tawara, T. Goto, Y. Watanabe, F. Matsumoto, and S. Kuwahara. 2000. Efficacy of FK463, a new lipopeptide antifungal agent, in mouse models of disseminated candidiasis and aspergillosis. Antimicrob. Agents Chemother. 44:614–618.
- Joshi S. Medicinal plants. 1st ed. Delhi: Oxford and IBH Publisher; 2000.
- Kerr DNS, Pearson DT, Read AE. Infection of ascitic fluid in patients with hepatic cirrhosis. Gut 1963; 4:394–8.
- Kokate C.K., Purohit A.P., and Gokhale S.B. (2006) Pharmacognosy; 23 ed., Niraliprakashan: pp:- 493-497
- Krencker E. Bacterium coli commune alsSepsiserreger in 2 fallen von abdominalerkrankungen. Munchen Med Wschr 1907; 54: 2095.
- Krishnamoorthy V, Madalageri MB. Bishop weeds (*Trachyspermumammi*): An essential crop for North Karnataka. Journal of Medicinal and Aromatic Plant Sciences. 1999; 21(4):996-998.
- Marchesi J, Shanahan F. The normal intestinal microbiota. CurrOpin Infect Dis 2007; 20: 508–13.
- Mendham, J., Denney, R. C., Bames, J. D., & Thomas, M. (2002). Vogel's Text Book of Quantitative Chemical Analysis, 6th eds.
- OCDE 423, 2001: OECD GUIDELINE FOR TESTING OF CHEMICALS Acute Oral Toxicity, Pg. 01-14.
- R Jain and SK Jain, Total Phenolic Contents and Antioxidant Activities of Some Selected Anticancer Medicinal Plants from Chhattisgarh State, India.Pharmacologyonline, 2011,2: 755-762
- Ranjan B, Manmohan S, Singh SR, Singh RB. Medicinal uses of *Trachyspermumammi*: a review. Pharmacogn Rev. 2012; 6(11): 56–60.
- Rex, J. H., Nelson, P. W., Paetznick, V. L., Lozano-Chiu, M., Espinel-Ingroff, A., &Anaissie, E. J. (1998). Optimizing the correlation between results of testing in vitro and therapeutic outcome in vivo for fluconazole by testing critical isolates in a murine model of invasive candidiasis. Antimicrobial agents and chemotherapy, 42(1), 129-134.
- Rimola A, Garcia-Tsao G, Navasa M, et al. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. J Hepatol 2000; 32: 142–53.
- Rodriguez-Tudela, J. L., Arendrup, M. C., Barchiesi, F., Bille, J., Chryssanthou, E., Cuenca-Estrella, M., &Fegeler, W. (2008). EUCAST Definitive Document EDef 7.1: method for the determination of broth dilution MICs of antifungal agents for fermentative yeasts: Subcommittee on Antifungal Susceptibility Testing (AFST) of the ESCMID European Committee for Antimicrobial Susceptibility Testing (EUCAST)∗. Clinical Microbiology and Infection, 14(4), 398-405.
- Sangeetha, J., &Vijayalakshmi, K. (2011). Determination of bioactive components of ethyl acetate fraction of Punicagranatum rind extract. Int J Pharm Sci Drug Res, 3(2), 116-122.
- Singal AK, Salameh H, Kamath PS. Prevalence and in-hospital mortality trends of infections among patients with cirrhosis: a nationwide study of hospitalised patients in the United States. Aliment PharmacolTher 2014; 40: 105–12.
- Slaoui, M., &Fiette, L. (2011). Histopathology procedures: from tissue sampling to histopathological evaluation. Drug Safety Evaluation: Methods and Protocols, 69-82.
- Soni R, Sharma G, Asuja DN. Essential oil yield pattern and antibacterial and insecticidal activities of *Trachyspermumammi*and *Myristicafragrans*. Scientifica. 2016; 2:1-7.

- Teixeira-da-Cunha, M. G., Gomes, R. N., Roehrs, N., Bozza, F. A., Prescott, S. M., Stafforini, D., & Castro-Faria-Neto, H. C. (2013). Bacterial clearance is improved in septic mice by platelet-activating factor-acetylhydrolase (PAF-AH) administration. PloS one, 8(9), e74567.
- Thangam C, Dhananjayan R. Anti-inflammatory potential of the seeds of *CarumCopticum*Linn. Indian J Pharmacol. 2003 Nov 1; 35(6):388–91.
- Tonekaboni H. Tohfatolmomenin. 1st ed. Tehran: Nashreshahr Press; 2007.
- Veerapur, V. P., Prabhakar, K. R., Parihar, V. K., Kandadi, M. R., Ramakrishana, S., Mishra, B., &Unnikrishnan, M. K. (2009). Ficusracemosa stem bark extract: a potent antioxidant and a probable natural radioprotector. Evidence-Based Complementary and Alternative Medicine, 6(3), 317-324.
- Zhishen, J.; Mengcheng, T.; Jianming, W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. **1999**, 64, 555-559.