Collection and identification of *E.coli* and check its antibiotic activity against medicinal plant that is Neem, Turmeric and Parijat

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ABSTRACT

A present study was conducted to isolate, identify and characterize the *E.coli* bacteria from turbid water and check its antibiotic activity against Neem, Turmeric, Parijat Plant. From this plant collect plant leaf air dried and crushed it and prepaid fine powder and applies on whatsman filter paper and observes its zone of inhibition against E.Coli. Maximum zone of inhibition was seen under Neem plant contaning disc.

Key words- E.coli, Medicinal plant, zone of inhibition.

INTRODUCTION

For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. Now a days , the use of photochemical for pharmaceutical purpose has gradually increased in many countries according to world health organization(WHO) medical plants would be the best source to obtain a variety of drugs. Antibiotic resistance has become a serious and widespread problem in developing countries , both in hospitals and the communality causing high mortality each other (Gyles C., 2011). In recent years antimicrobial properties of medicinal plant are eing increasingly reported for different parts of the word (Grosvenor *et al*.,1995; Ahmad *et al*., 2001; Abu-Shanab *et al*., 2008). One of the objectives of our research group is to investigate the potential antibacterial properties of medicinal plants . In the present study , we used three plants that have the potential to be use as antibacterial agents against *E.coli* to conduct antibacterial activity assays against *E.coli* isolated from turbid water. The three plants used in this study. Neem , Turmeric , Parijat . The vast majority of modern medications were derived originally from ancient herbal traditions. The practices of pants based traditional medicine are found on hundreds of years of belief and observations, which predate the development of modern medicine medicinal plant have been used for centuries as remedies for human diseases as they contain components of therapeutic value. Three are numerals plant natural products which have antifungal and antiportozoal activities that could be used either systemically or locally (Cowan, 1999). Inappropriate usage of antibiotics is the most influential factor of antibiotic resistance and the global emergence of multi-drug resistant bacteria in increasing limiting the effectiveness of current drug and significantly causing treatment failure (Djeussi *et al*., 2013). Medicinal plants have been tested for biological, antimicrobial and hypoglycemic activity. They have also tested for antiulcerogenic, antihelminthic, hepatoprotective, analgesic, antipyretic, antileishmania and insecticidal activity (Doughari *et al*., 2008). A lot of supplementary treatments strategies have been tried. Current social trends in healthcare show a definite movement towards the use of natural remedies like medicinal plants and away from chemotherapeutic regimens (Daniyan *et al*., 2008). In the present study, and attempt has been made with the antimicrobial activity of extracts of certain selected medicinal plants on some human pathogenic bacteria.

MATERIALS AND METHODS

Collection of bacteria

We accrued bacteria from turbid water in sterilized container and then inoculated on prepared nutrient agar plate for isolation after which we isolated the microorganism. Then this microorganism inoculated in MacConkey agar and incubates at 37°C for 24-48 hours after which we isolate pure microorganism.

Bacterial colony identification and external morphology study

The usage of the spread plate method the bacterial colony identity and external morphology become studied for which nutrient agar media turned into organized. Consequently one hundred ml of Nutrient agar Media was prepared for four Petri plates. The NA media become autoclaved and then poured in four Petri dishes which have been additionally sterilized via autoclave. Then the serial dilutions of 10^{-2}, 10^{-4}, 10^{-6}, and 10^{-8} were selected and from that 0.1 ml of way of life turned into transferred from each serially diluted test tubes and spread at the Petri plates with the aid of the spreader. Then the Petri dishes were stored within the incubator for 37^0 Celsius for 24 hrs for the incubation and boom of micro organism. After 24 hrs of incubation the Petri dishes were taken out of the incubators and the subsequent bacterial outside morphology have been studied.

Pure culture isolation of bacteria

Well developed and separated colonies which were recognized on the MacConkey agar plates had been marked after which those separated colonies had been chosen and through the assist of inoculating needle the colonies had been transferred and streaked separately on test tubes having nutrient agar slants for the growth of the single colonies of bacterial cultures from the mixed culture of microorganism. That turned into grown within the Petri plates. The take a test tubes had been marked after the strains of chosen colonies from Petri plates and had been left inside the incubator at 37° Celsius overnight for growth and incubation. After incubation of the pure cultures in a single day special single species of bacterial culture slants evolved in the take a test tubes which have been in addition picked and purified.

Microscopic study

The pure cultures of various colonies that were received in test tubes were put for gram staining for more unique identification of the colonies. The gram staining changed into accomplished in laminar airflow hood. For this purpose the slides were taken from slide rack. The slides have been washed with ethanol. Then each colony changed into marked on the slides. Then with the assist of inoculating needle the loopful strains had been picked from each take a look at tube and made a smear at the slides and warmth constant. The slides were then taken in the staining room for staining the smears. Then smears were stained in following steps a) First applied crystal violet on each slides and stored for 30 secs. b) Distilled water wash. c) Iodine on the slides as mordant (1 min) then 95% alcohol washes and then washed with distilled water. d) Safranin becomes applied at the slides after which Washed with distilled water and f) the slides air dried. The entire gram staining technique was done following the Christian Gram technique (Collee *et al.,* 1996).

Identification of Bacterial Isolates through BiochemicalTest

The isolated from turbid water turned into recognized up to familiar degree primarily based on morphological cultural and biochemical tests as laid out in Bergey's guide of Determinative Bacteriology (Gilman, 1957). Biochemical take a test at became finished as counseled with the aid of (Holt et al., 1994) which protected tests as like Gram's stain, IMViC reaction, catalase test, starch hydrolysis test oxidation fermentation test, Nitrate reduction, Gelatin hydrolysis test, Urea hydrolysis test, Dehydrogenase test, Citrate utilization test, Indol production test, Triple Sugar Iron (TSI) test, Carbohydrate fermentation test. For isolated bacterial identification as per to Bergey's manual we performed test. IMViC test show Indole, Methyl red, Voges-proskauer (VP) and citrate. Then Gelatinase ,Nitrate ,Catalase ,Oxidase , H_2S.

Collection of plant

The three medicinal plants tested in this study are shown in table .This are easily available in environment. We collected the fresh plants leaves Neem, Turmeric, Parijat.

Sr.No.	Scientific name	Family	Local name
(1)	Azadirachta indica	Mohogany	Neem
(2)	Curcuma longa	Ginger	Turmeric
(3)	Nyctanthes arbor -tristis	Oleaceae	Parijat

Table no 1: Selected plant

Preparation of fine powder

First off we take a 3 medicinal plant leaves Neem, Turmeric, Parijat. Then this leaves washed with D.W. to dispose of the unwanted materials then air-dried and dry in underneath sunlight. The temperature of daylight is forty 42-43°c for 48 hour after which we scrub or grind this leaves .Then we discovered a fine powder. Then we sterilize this powder in warm air oven and store in sterile container.

Preparation of sterile disk

Whatman's filter out paper became punched into 5mm disc from and they sterilized each sterile disc. Precaution has been taken to prevent the flow of the solvent extract from the discs to the outer surface. The amassed powder extracts had been carried out in small quantities on disc.

Assay of antimicrobial activity using agar disc diffusion method

The sensitivity of different bacterial strains to the aqueous plant extracts was measured using a standard agar diffusion assay (Bauer *et al*., 1996). The 30 ml of nutrient agar became poured in to sterile petriplate, after solidification 100 µl of fresh culture of *E.coli* had been poured on the respective plates. After some time culture turned into discard from agar plate. Then take sterilized forceps and follow the disk carefully in center of agar plate .The plates had been incubated for 24-48 hour at 37°c. After incubation the diameter of inhibitory zone shaped around each discs were measured in cm and recorded.

RESULT and DISCUSSION

The aim of the present study was first upon collection of *E.Coli* and compares the antimicrobial activity of Neem, turmeric, parijat leaves. On the basis of cultural character, Morphological character (colony color, shape and size) and biochemical character isolated bacteria was identifying. Following character was compare with 'BERGEY'S. Isolated bacterial result was given in following tables.

Sr.No.	Test	RESULT
1)	Gram	Negative (-ve)
2)	Spore	Non- sporeforming
3)	Motility	Motile

Table no 2: Microscopic study

Sr.No.	Test	Result
1)	Indol	Positive (+ve)
2)	Methyl Red	Positive (+ve)
3)	Voges-proskauer (VP)	Negative (-ve)
4)	Citrate	Negative (-ve)
5)	Gelatinase	Negative(-ve)
6)	Nitrate	Positive(+ve)
7)	Catalase	Positive(+ve)
8)	Oxidase	Negative(-ve)
9)	H_2S	Negative(-ve)
10)	Glucose	Positive(+ve)
11)	Lactose	Positive(+ve)
12)	Sucrose	Positive (+ve)
13)	Galactose	Positive(+ve)

Table no. 3: Biochemical test

After analyzing the colony morphology on nutrient agar medium, colony morphology changed into additionally studied on the selective media. After the secondary identification on selective media, all samples were tested microscopically right here the shape size and motility changed into checked and the gram staining technique are followed. After observe above test now we conformed isolated bacteria culture was *E.Coli*.

Three plant medicinal selected that is, Neem, Turmeric, Parijat had been plant leaves collected to test the antimicrobial activity at *E.coli* isolated from turbid water sample by the "agar well diffusion technique" and the diameter area of inhibition become measured in mm. Antimicrobial activity of selected plant on the *E.coli* is given as in table 2. In present study maximum zone of inhibiton was observed in Neem plant leaves against *E.coli* (2.2 mm). And lowest zone of inhibition was observed against Turmeric plant leaves (1.5 mm). Average zone of inhibition was seen in against Parijat plant leaves (1.3 mm). Various researchers have worked in exploring the antimicrobial potency of leaf extracts against infectious bacteria. (Suree and Pana, 2005) found ethanolic extracts and essential oil of Zingiber officinale and Myristica fragrans to be effective against the Enterobatericae and concluded that the varying degree of sensitivity of microbes may be due the nature and combination of the phytocompounds present in the extracts. (Ahmad and Aqil, 2007) concluded that ethanolic extracts of garlic did not have anti-*E.coli* or anti-*Shigella* action. The results obtained from the present study provide evidence that Neem, Turmeric, Parijat plant exhibit antibacterial activities against isolated *E.Coli* strain, which suggests that they may be clinically useful. One of the efforts in this research is focused on the use of medicinal plants, which are widely available resources, less if no side effects, less expensive and have shown antimicrobial properties (Rubens *et al*., 2015) . After observing zone of inhibition then calculate the percentage of inhibition by using following formula.

$$\text{Percentage of inhibition} = \frac{\text{Zone of inhibition}}{\text{Diameter of colony}} \times 100$$

Table no. 4:

Sr.No	Medicinal plant	Diameter	Zone 0f inhibition	% Of inhibition
1)	Neem	1.3	2.2	169.2307
2)	Turmeric	1.2	1.5	125
3)	Parijat	1.1	1.3	118.1818

Biochemical test

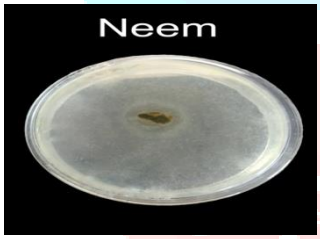

Figure 1: Neem

Figure 2:Turmaric

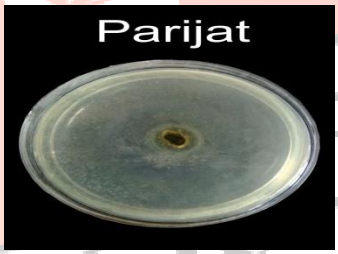

Figure 3: Parijat

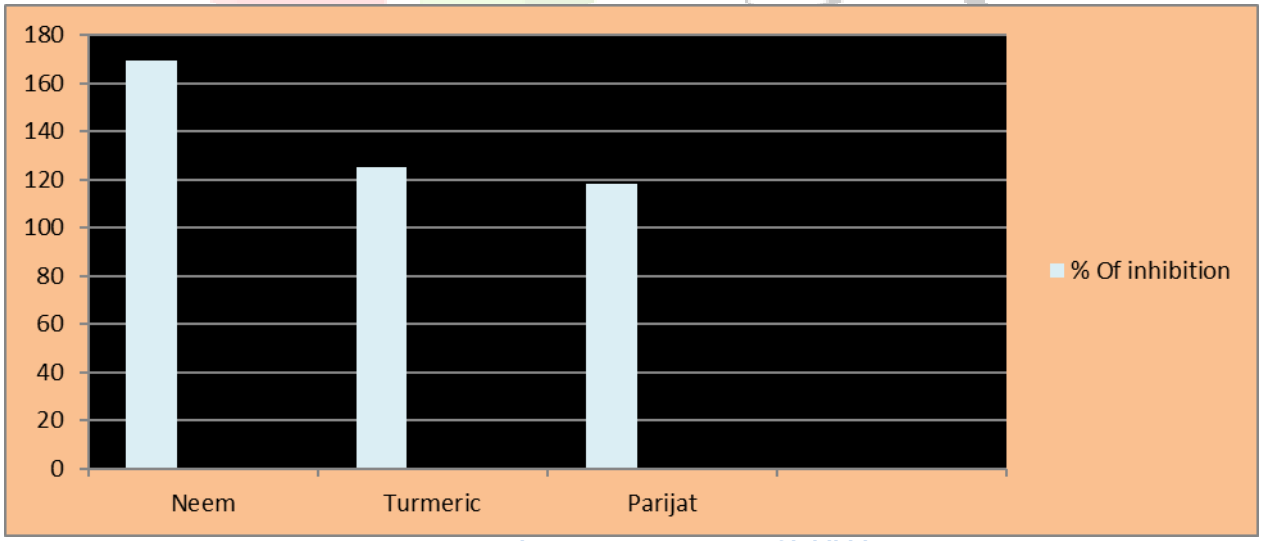

Figure 4. Percentages of inhibition

CONCLUSION

The current study has identified *E.coli* bacteria from turbid water and checks its antibiotic activity against Neem, Turmeric, Parijat plant. It was investigated that Neem plant showed maximum antibacterial activity against *E.coli*. Hence here we conculded that for controling *E.coil* infection use Neem plant leaves.

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REFERENCE

1) Abu-Shanab BA, Adwan G, Adwan KM, Abu-Shanab F. Efficacy of aqueous and ethanol extracts of some Palestinian medicinal plants for potential antibacterial activity. Islam Univ J Gaza 2008; 16(2): 77-86.

2) Ahmad, I.; Aqil, F. (2007). In vitro efficacy of bioactive extracts of 15 medicinal plants against ESbL-producing multidrug-resistant enteric bacteria. *Microbiol. Res.*, 162: 264-275.

3) Ahmad I, Beg AZ. Antimicrobial and phytochemical studies on 45 Indian medicinal plants against multi-drug resistant human pathogens. J Ethnopharmacol 2001; 74(2): 113-23.

4) Bauer AW, Kirby WMM, Sherris JC, Turck M: Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology. 1996, 45: 493-496

5) Cowan MM. Plant products as antimicrobial agents. Clin Microbiol Rev 1999; 12(4): 564-82.

6) Collee, J.G., Miles, R.S. and Watt, B. 1996. Test for the identification of bacteria. In: Collee, J.G., Faser, A.G., Marmion, B.P., Simmons, A. (Eds). Mackie and McCartney practical medical microbiology, 14th edn. Churchill Livingstone, London. Pp. 13 -145.

7) Daniyan, S.Y. and H.B. Mahammad. (2008). *Afri. J. of Biotec.* 7(14): 2451-2453.

8) Djeussi DE, Noumedem JA, Seukep JA, Fankam AG, Voukeng IK, Tankeo SB, et al. Antibacterial activities of selected edible plants extracts against multidrug-resistant Gram-negative bacteria. BMC Complement Altern Med 2013; 13: 164.

9) Doughari, J.H. and J.S. Obidah. (2008). *Inte J. of Inte. Bio.* 3 : 111-117.

10) Gilman J.C. second ed. Oxford and IBH Publishing Company; New Delhi, India: 1957. A Manual of Soil Fungi.

11) Grosvenor PW, Supriono A, Gray DO. Medicinal plants from Riau Province, Sumatra, Indonesia. Part 2: antibacterial and antifungal activity. J Ethnopharmacol 1995; 45(2): 97-111.

12) Gyles C. The growing problem of antimicrobialresistance. *Can Vet J* 2011; 52(8): 817-20.

13) Holt JG, Krieg NR, Sneath PHA, Staley JT. and Williams ST. 1994. Bergey's Manual of Determinative Bacteriology, 9th ed. Williams and Wilkins, Baltimore, Maryland.

14) Rubens DM, Constantin OO, Moevi AA, Nathalie GK, Daouda T, David NJ, et al. Anti Staphylococcus aureus activity of the aqueous extract and hexanic fraction of Thonningia sanguinea (Cote ivoire). Int J Pharmacogn Phytochem Res 2015; 7(2): 301-6.

15) Suree N and Pana L. (2005) Sci. Tech. J. 5(3): 527-538.