Journal Of Harmonized Research (JOHR)

Journal Of Harmonized Research in Pharmacy
5(1), 2016, 36-39

ISSN 2321 – 0958

Original Research Article

ISOLATION AND INVESTIGATION OF ANTIMICROBIAL ACTIVITY OF THE LEAVES OF *THEA SINENSIS*

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Abstract: In the present study the ethanolic extract and isolated caffeine obtained from the *Thea sinesis* were tested for antibacterial activity against 13 Bacterial strains both gram positive and gram negative by determining minimum inhibitory concentration (MIC) and zone of inhibition (ZOI). MIC values were compared with standard chloramphenicol in concentrations of 30 & 100 µg/ml. The result of MIC & ZOI and its competition with standard antibiotics it evident that the ethanolic extract is active against both gram positive and gram negative bacteria. Further the isolated caffeine was characterized by different analytical techniques like TLC, UV-VIS spectroscopy & HPLC and compared with the slandered.

Key Words: - *Thea sinesis*, ZOI, MIC

Introduction: *Thea sinesis* belongs to family Theaceae (Ternstroemiaceae). Traditionally the infusion of tea were used as stimulant, diuretics, astringent, blood purifier & due to its peripheral action on the heart circulatory system & autonomic functions it was also used for lowering of the risk of ischemic heart diseases in older man.[1] The infusion of tea contains in addition to caffeine a mixture of polyphenols including epigallocatechin-3-gallate possessing strong antioxidant & free radical scavenging properties.[2] The present study involves determination of antibacterial activity obtained from *Thea sinesis*.

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Received on: January 2016
Accepted after revision: March 2016
Downloaded from: www.johronline.com

Material and Method: Extraction: - The dried tea powder was purchased from local market and subjected to soxhlet extraction with ethanol for 6 hrs. The extract was filtered on hot condition and concentrated in vacuum under reduced pressure and dried in desicator and further purified by recrystalisation from methanol. The caffeine was isolated from the extract by chemical method [3] .The % yield of the extract was 16.82% (w/w) & the % yield of caffeine was 1.4% (w/w).

Charecterization of Isolated Caffeine: -The isolated caffeine was identified by different analytical methods such as TLC, UV-VIS spectroscopy, HPLC, melting point and compared with standard. In TLC R_f = 0.70 ($CHCl_3$:MeOH:: 9:1, silica gelG)[4]; UV: λ_{max} (MeOH)=272.5nm; HPLC: R_t=2.97 min using MeOH as mobile phase, column C18 (250×4.6 mm), flow rate of 1 ml/min, λ_{max} 272nm by UV detector and melting point 235^0c [5]respectively. These all data were identical in every respect with the authentic sample of caffeine.

Determination of Minimum Inhibitory Concentration (MIC): -The molten nutrient media containing various concentrations of the extract (0, 20,40,60,80,100 & 120 µg/ml) and isolated caffeine (0, 10, 20, 30, 70 & 100 µg/ml) were poured and solidified onto sterile 100 mm petridishes to give sterile nutrient agar plates with varying dilutions of the extract and isolated caffeine. Then these plates were kept in refrigerator (4^0c) for 24 hrs. for uniform diffusion of the extract into the nutrient agar media. The plates were then dried at 37^0c for 2 hrs. before spot inoculation[6]. One loopful of an overnight grown peptone water culture of each test organism was placed in petridish marked by checkerboard thechnique[7]. The spot inoculated plates were inoculated at 37^0c for 24 hrs.and MIC values were obtained.

Determination of Zone of Inhibition By Disc Diffusion Method[8]: -Here we have taken pure chloramphenicol as a standard antibiotic for comparing of the result. Two sets of dilutions (30 and 100 µg/ml) of tea extract and chloramphenicol (30 and 100 µg/ml) were prepared in double distilled water in Mc cartney bottles. Sterile nutrient agar plates were prepared and incubated at 37^0c for 24 hrs. to check any sort of contamination. Two sterile filter paper discs (whatman no.1) of 6 mm diameter were soaked in two different dilutions of the crude extract and placed in appropriate positions on the surface of the flooded plates, marked as quadrants at back of the petridishes. The petridishes were incubated at 37^0c for 24 hrs. and the diameter of zone of inhibition measured in mm. similar procedure was adopted for the pure chloramphenicol and the corresponding zone diameter were compared accordingly. The experiment was repeated in triplicate and average values were written in following tables.

Table-1: Determination of MIC of ethanolic extract of Tea powder (Agar dilution method)

Name of Bacteria	Growth in nutrient agar containing different concentration of extract in µg/ml.						
	0	20	40	60	80	100	120
1. *S. aureus* NCTC- 65 H	+	+	+	+	+	-	-
2. *S. aureus* ATCC -29737	+	+	+	+	+	+	-
3. *S. aureus* ML -59	+	+	+	+	-	-	-
4. *S. aureus* ML -125	+	+	+	+	+	-	-
5. *B.subtilis -70*	+	+	+	+	-	-	-
6. *B.pumilus* NCTC -8241	+	+	+	+	+	-	-
7. *B.brevis* NCTC- 7096	+	+	+	+	+	+	-
8. *V.cholerae -23*	+	+	+	+	-	-	-
9. *V.cholerae- 564*	+	+	+	+	+	-	-
10. *V.cholerae -1002*	+	+	+	+	+	+	-
11. *E.coli* HB -101	+	+	+	-	-	-	-
12. *E.coli* C -600	+	+	+	+	-	-	-
13. *E.coli* R -832	+	+	+	+	-	-	-

'0' control (without extract); '+' Growth; '-'No growth

TABLE -2: - Determination of MIC of isolated caffeine from Tea powder (Agar dilution method)

Name of Bacteria	Growth in nutrient agar containing different concentration of isolated caffeine in µg/ml.					
	0	10	20	30	70	100
1. *S. aureus* NCTC- 65 H	+	+	+	+	+	+
2. *S. aureus* ATCC- 29737	+	+	+	+	+	+
3. *S. aureus* ML -59	+	+	+	+	+	-
4. *S. aureous* ML- 125	+	+	+	+	+	-
5. *B.subtilis*- 70	+	+	+	+	+	-
6. *B.pumilus* NCTC -8241	+	+	+	+	+	+
7. *B.brevis* NCTC -7096	+	+	+	+	+	+
8. *V.cholerae*- 23	+	+	+	+	+	+
9. *V.cholerae* -564	+	+	+	+	+	+
10. *V.cholerae*- 1002	+	+	+	+	+	-
11. *E.coli* HB- 101	+	+	+	+	+	-
12. *E.coli* C- 600	+	+	+	+	+	+
13. *E.coli* R- 832	+	+	+	+	+	-

'0' control (without extract); '+' Growth; '-'No growth

Table-3: - Determination of zone of inhibition produced by the ethanolic extract and its comparison with chloramphenicol.

Name of Bacteria	Ethanolic Extract (µg/ml)		Chloramphenicol (µg/ml)	
	100	200	100	200
1. *S. aureus* NCTC- 65 H	10	22	6.5	11
2. *S. aureus* ATCC- 29737	12	24	7	113
3. *S. aureus* ML -59	12	20	7	9.5
4. *S. aureous* ML- 125	10	22	6.5	10
5. *B.subtilis*- 70	20	35	6.8	12
6. *B.pumilus* NCTC -8241	23	39	7	11
7. *B.brevis* NCTC -7096	22	33	6.5	14
8. *V.cholerae*- 23	27	40	7.8	19
9. *V.cholerae* -564	30	42	9.4	18
10. *V.cholerae*- 1002	26	40	10.2	20
11. *E.coli* HB- 101	22	36	11.3	22
12. *E.coli* C- 600	20	32	10.5	21
13. *E.coli* R- 832	22	38	12	23

Values of zone of inhibition in (mm), tests were done in triplicate.

Result: - MIC of the ethanolic extract and isolated caffeine were found to be varying between 20-120 µg/ml and 10-100 µg/ml respectively, with respect to most of the test bacteria. The MIC of ethanolic extract for bacterial strain E.coli HB-101 was found to be 60 g/ml, for V.cholerae-23, B.subtilis-70, and for S.aureus ML-59 were found to be 100 g/ml. The MIC of isolated caffeine for V.cholerae-1002, E.coli HB-101, B.subtilis-70 and S.aureus ML-59 were found to be 100 µg/ml. The result of ZOI of the extract was compared with standard antibiotic chloramphenicol (100 & 200 µg/ml) and

recorded. The antibacterial efficacy of extract of *Thea sinesis* was found to increase in the following order against different tested bacterial strains- E.coli R-832, E.coli HB-101, V.cholerae-23, B.brevis NCTC-7096, and S.aureus ATCC-29737.

CONCLUSION: - The result of MIC & ZOI values and its competition with standard antibiotic chloramphenicol it evident that the ethanolic extract is active against gram positive and gram negative bacteria. The antibacterial properties of the plant may be attributed to the combined effect of the present chemical groups in the extract. So to know the phytoconstituent responsible for antibacterial activity should be further investigated.

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