VALIDATION OF ANTI-BACTERIAL EFFECT OF AMALTAS (Cassia *fistula,* METHANOLIC EXTRACT) AGAINSTSELECTED BACTERIAL STRAINS

F. Zubair, N. Riaz, I. Khalid
Department of Zoology, University of Sargodha

ABSTRACT: Increasing prevalence of antibiotic resistance necessitates the development of more potent natural product that costs no adverse effects to human health. Purpose of this study is to verify the medicinal efficacy of selected plant extract (*Cassia fistula)* against selected bacterial strains. For this purpose the methanolic extract of *C. fistula* (Amaltas) was employed at different concentrations, temperature and pH conditions to study its antimicrobial properties against *Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Bacillus cereus*. Results revealed that *C. fistula* showed maximum zone of inhibition (2.6mm±1.1) at 250mg/ml against *B.subtilis* while minimum zone (0.6mm±0.00) was observed against *Staphy.aureus* at 150mg/ml. The results for varying pH conditions reveled that *C. fistula* exhibits maximum inhibition (1.5mm±0.2) of *B. subtilis* at pH-9 (concentration: 150mg/ml) while at 250mg/ml the observed zone measurement was maximum against *Staph.aureus* at pH-9. The results at different temperature range depicts that *C. fistula* is effectively controlling the growth of *B.subtilis* at 120ºC (250mg/ml, zone of inhibition: 1.7mm±0.2). All the zones of inhibition were found significant according to the One way ANOVA. This results of current study strengthens the fact regarding antibacterial potential of plant extracts and proves that increasing incidence of antibiotic resistance can be reduced by introduction of more natural antibacterial agents other than chemicals.

Keywords: Antibacterial activity, *C. fistula, E.coli, Staph.aureus, B.subtilis, B. cereus*

INTRODUCTION

In survival of humans and animals, role of plants on this planet is irreplaceable, to cure and treat different forms of diseases, plant derived products are in common use (Oladeji, 2016). Plants have beneficial ingredients which are used in research and their products comprise of wealth of components are utilized in medicine, whether pharmacopoeial, non-pharmacopoeial, or synthetic pharmaceuticals (Yuan, *et al*., 2016). One of the reasons to use natural products is that they are less harmful to human body and all these products also prove to be compatible with human physiology (Oladeji, 2016).

Precursors of plants used for production of valuable drug is termed as medicinal plant (Sofowora *et al*., 2013). Use of plants for attaining medicinal purpose termed as alternative medicine (Hassan, 2012). *Cassia fistula* also known as golden shower is a deciduous plant, with yellow flowers with compoundleaves, native to India and Sri Lanka used in western drugs as a model. Ornamental Fast growing tree with giant flower part belongs to legumes family while in some areas also called Indian laburnum (Dutta and Madharia, 2012). In Ayurveda and Chinese traditional Medicine, extracts of *C. fistula* is used to treat diseases and earned name Yellow shower(Mwangi, 2021).

In old days it is used in traditional healing practice in crude form. Due to medicinal properties of this plant, itis used in modern world excessively. Studies enlighten their importance due to their biological properties. Some species of *Cassia* act as a laxative (Dutta and Madharia, 2012). *C.fistula* is an imperative source of naturally occurring bioactive compounds. Both in vivo and in vitro extracts, polyphenolics are abundantly found and these polyphenolics prove very important, nontoxic and chemo preventive against certain oxidative stresses. *C. fistula*extract biological effects are due to presence of primary and secondary metabolites. Analysis cleared that primary metabolite attain from seeds, fruits, pollen, pod and leafand seeds are richer in fatty acid contents. Various components of plants such as Alkaloids, glycosides, tannins and Flavoids comprises antimicrobial activity. Properties of these components also lessens the burden of chemical remedies (Yadav and Agarwala, 2011).

In Indian system of medicine, *C. fistula* has great therapeutic implication. Phytochemicals present in plant extract exerts different actions. Over the past few years, importance of this plant increases due to its properties, Antipyretic and action against inflammation all are its effects. This plant also possesses antioxidant properties. Lipid oxidation also done due to its antioxidant components. Inhibition is by the initiation or propagation of oxidizing chain reactions. Important phytochemicals present in various goods like Fruits and vegetables are reported helpful in treatment of genetic dysfunction diseases and infectious disease (Ugboko *et al.,* 2020).

This study aim to support the fact of using herbal options for treatment of bacterial associated infections by researching the efficacy of methanolic extract of *C.fistula* against selected strains of bacteria and

standardization of results at variable range of temperature and pH conditions.

MATERIALS AND METHODS

Preparation of methanolic extract: About 1000gm powder of *C.fistula* and 1600ml of methanol was placed in soxhlet apparatus at 60^0C for 10hours then material was mix in methanol. Solvent containing extract material was evaporated in rotary evaporator. Slow rotation with 50^0C of water bath of rotary evaporator, methanol was evaporated. Further extract was dried by evaporation of minute amount of methanol at room temperature. At the end about 120gm of *C. fistula* extract is stored in Pyrex jar for experimental purpose.

Bacterial strains and Inoculums preparation: Four strains were isolated and identified in microbiology lab, department of zoology,University of Sargodha. These strains were *E. coli, B. subtilis, B. cereus* and *staph. aureus.* By using specific growth media, bacterial strains were cultured in lab, at specific time and temperature. For *Staphy. aureus, E. coli* and *B.subtilis* nutrient agar media was used while *B. cereus* was cultured on MRS broth media. After culturing strains were incubated at 37^0C for 24 hours (Shaikh *et al*., 2019).

Inhibition zone assay: By using the technique of inhibition zone assay, methanolic extract of *C.fistula* was tested on selected bacterial strains. Antibacterial potential of these medicinal plants was observed on selected bacterial strains. In first step, colonies of bacterial strains were picked from already cultured bacterial strains. Then dissolves properly into tubes by using water for injection. Then these diluted strains further used in experiment. On prepared Petri dishes comprise of specific media, dilution was spread using spreader, bacterial lawn was created. After drying, sterile cork borer was used to proceed for well diffusion method. Diameter of wells was 6.00mm. Methanolic extract was diluted according tothe required concentration. Then 100 µL of diluted extract was poured in wells. On plate there was control group that contain no plant extract material and plates incubated at 37 ^{0}C for one day. After 24 hours minimum inhibitory zones were measured by using Vernier caliper. This whole protocol was performed in triplicate to verify the results.

Minimum inhibitory concentration (MIC) assay: After screening test of crude plant extract, methanolic extract was applied on strains. Five concentrations of methanolic plant extracts (150mg/ml, 180mg/ml, 200mg/ml, 220mg/ml 250mg/ml) was tested for zone of inhibition.

Effect of Temperature on antibacterial activity of plant extract: Effect of temperature was investigated by immersing test tubes containing extract material in a water bath. The temperature of the plant extract was changed by altering the water bath temperature. Temperatures of around 40, 60, 80, 100, and 121 °C were varied before extract was applied to bacterialstrains. In the first step, the concentration of 150 mg/ml extract was adjusted at different temperatures of 40, 60, 100, and 121°C and then applied to strains using the well diffusion method. Various concentrations of methanolic plant extractwere adjusted for 30 minutes at various temperatures.

Effect of pH on antibacterial activity of plant Extract: Effect of pH was tested by altering the pH of solution. 3pH, 5pH, 7pH, 9pH and 11pH values were used on two different concentrations (150mg/ml and 250mg/ml). For altering the pH of solution, NaOH and HCl were used. This experiment was repeated three times for each methanolic extract.

Statistical Analysis: In order to compare the mean values of zone of inhibition of methanolic extract of *C. fistula* at different concentration, different temperature and different pH, One way ANOVA followed by Tukey's test was applied.

RESULTS/DISCUSSION

This study evaluates the antimicrobial activity of medicinal plants. Traditionally, herbs have been utilized to cure illnesses and to restore and strengthen bodily systems (Aslam and Ahmad, 2016). To treat the infectious diseases, antibiotics are used, but antibiotic treatment is not only costly, but it also carries the danger of bacterial resistance to antimicrobial medicines as well as side effects such as acidity, burning sensation, and damage to the normal fauna of the intestine. So, scientist moves towards the use of plants (Nagpal, *et al.,* 2011).

In this study plant extract is used to inhibit the growth of test strains. For alltest strains, minimum inhibitory concentration was 150mg/ml. Extract showed zone of 1.1mm in size for *B. cereus* and *E.coli* at minimum inhibitory concentration (Table 1). Zone of inhibition for *B. subtilis* was 0.8mm. While zone of inhibition for *Staph. aureus* was 0.6mm at 150mg/ml. So, methanolic extract of *C. fistula* was found more effective against *B. cereus* followed by *E.coli, B. subtilis* and *Staphy. aureus*. A previous study demonstrated that the antimicrobial activity depends on the contents of phenolic components of the plantextracts. High amounts of phenolic group in the aerial parts of *C. fistula* implied that these components may be the active compounds, which may be responsible for the antibacterial activity (Seyyednejad *et al*., 2014). In another study the researchers observed that the extracts from leaves of *C. fistula* had broad-spectrum effect and therefore could be employed in the management of infectious diseases (Bhalodia. and Shukla, 2011).

During current study, antimicrobial efficacy of *C. fistula* was tested against selected strains of Gram positive bacteria and Gram-negative bacteria using its methanolic extract. The results showed that extract has noticeable result against *E. coli* and *B. cereus.* Similar results were reported by Seyyednejad *et al.,* (2014). They reported that minimum inhibitory concentration of extract, zone of inhibition for *E. coli*

was found maximum (26mm) in diameter. Overall, it is concluded that methanolic extract of *C. fistula* was proved effective against *E. coli.*

In another study, the antibacterial activity of *C. fistula* was reported at 3mg/ml to 75mg/ml against *B. cereus, Staph. aureus and E. coli* (Hamad *et al.*, 2017). The results of present study showed maximum inhibitory zones against the *Staph. Aureus*. The antibacterial activity of methanolic extract of *C. fistula* was also checked by heating extract solution at different temperature and altering pH at minimum and maximum concentrations. Table 2 shows the results of methanolic extract at150mg/ml concentration, by treating solution at different range of temperature. For 150mg/ml, at 40 °C, *B. cereus, B.subtilis* and *Staphy. aureus* showed minimum level of zone of inhibition. Extract showed maximum results against *E. coli.* Then further heating of extract solution results in varied level of zone of inhibition for all test strains. At 60°C, temperature extract showed maximum results against the *B. cereus.* Similarly at the temperature of 80°C, and 100 °C, maximum zone of inhibition were observed against *B.cereus*. But at the highest temperature of 121°C, extract showed maximum results against *B. subtilis*.

Table 3 indicated the results of methanolic extract of *C. fistula* at 250mg/ml, applied on bacterial strains at different temperature. Almost all bacterial strains showed zone of inhibition of diameter about 1.7mm at concentration of 250mg/ml while for *E. coli* zone of inhibition was maximum at 80°C. So, by treating the methanolic extract of *C. fistula* at high temperature, it will increase its antimicrobial property. In Table 4 and Table 5, antibacterial activity of methanolic extract of *C. fistula* is reported by altering the pH of minimum and maximum inhibitory concentration .At 3Ph, maximum results was observed against the *E. coli.* The outcomes of this study are mentioned in the following tables.

Table 1: methanolic extract of *C. fistula* at different concentration.

Bacterial Strains	**Methanolic Extract of *C. fistula* at different Conc.**					**Control group**	***P*-Value**
	150mg/ml	**180mg/ml**	**200mg/ml**	**220mg/ml**	**250mg/ml**		
Bacillus cereus	1.1±0.1	1.3±0.1	1.6±0.2	1.2±0.2	1.6±0.0	0±00	.009*
Bacillus subtilis	0.8±0.1	1.0±0.1	1.3±0.2	1.5±0.3	2.6±1.1	0±00	.014*
Escherichia coli	1.1±0.1	1.3±0.1	1.9±0.0	0.8±0.2	2±0.1	0±00	.000*
Staphylococc usaureus	0.6±0.0	0.9±0.0	1.2±0.1	1±0.2	1.5±0.1	0±00	.000*

Mean ± SD (n=3). Values present in rows are variables. *P* values was found by using ANOVAand compared with significant ($P \leq 0.05$). Non-significant values are ($P \geq 0.05$).

Table 2: methanolic extract of *C. fistula* at 150mg/ml by treating different temperature.

Bacterial Strains	**Methanolic Extract of *C. fistula* at 150mg/ml bytreating different temperature.**					**Control group**	***P* Value**
	40°C	**60°C**	**80°C**	**100°C**	**121°C**		
Bacillus cereus	0.7±0.2	1.3±0.1	1±0.2	1.5±0.2	1.5±0.2	0±00	.001*
Bacillus subtilis	0.7±0.2	1±0.2	0.8±0.2	1.3±0.2	1.7±0.2	0±00	.001*
Escherichia coli	1±0.2	1±0.2	0.8±0.2	0.9±0.2	1.5±0.2	0±00	.013*
Staphylococcus aureus	0.7±0.2	1.2±0.2	1±0.2	1.3±0.2	1.5±0.1	0±00	.002*

Mean ± SD (n=3). Values present in rows are variables. *P* values was found by using ANOVAand compared with significant (*P*≤0. 05). Non-significant values are *(P*≥0. 05).

TABLE 3: methanolic extract of *C. fistula* at 250mg/ml by treating different temperature.

Bacteria lStrains	**Methanolic Extract of *C. fistula* at 250mg/ml bytreating different temperature.**					**Control group**	***P* Value**
	40°C	**60°C**	**80°C**	**100°C**	**121°C**		
Bacillus cereus	1.3±0.1	1.5±0.2	1.0±0.2	1.6±0.2	1.7±0.1	0±00	.002*
Bacillus subtilis	1.0±0.2	0.8±0.2	1.0±0.3	1.6±0.2	1.7±0.1	0±00	.001*
Escherichia coli	1.3±0.1	1.2±0.2	2.6±1.1	1.2±0.2	1.7±0.1	0±00	.035*
Staphylococcus aureus	1±0.2	1.3±0.0	1.0±0.2	1.6±0.2	1.7±0.1	0±00	.001*

Mean ± SD (n=3). Values present in rows are variables. *P* values was found by using ANOVAand compared with significant (*P*≤0. 05). Non-significant values are *(P*≥0. 05).

Table 4: methanolic extract of *C. fistula* at 150mg/ml by at different pH.

Bacterial Strains	**Methanolic Extract of *C. fistula* at 150mg/ml bytreating different pH**					**Control group**	***P* Value**
	3 pH	**5 pH**	**7pH**	**9 pH**	**11pH**		
Bacillus cereus	0.7±0.1	0.7±0.1	1.5±0.2	1.1±0.1	0.8±0.1	0±00	.000*
Bacillus subtilis	0.7±0.1	1.0±0.1	1.2±0.1	1.5±0.2	0.4±0.2	0±00	.000*
Escherichia coli	1.1±0.1	1.1±0.1	0.7±0.1	1.4±0.1	0.4±0.2	0±00	.000*
Staphylococcus aureus	0.6±0.1	0.9±0.2	1.2±0.1	1.4±0.2	0.7±0.1	0±00	.000*

Mean ± SD (n=3). Values present in rows are variables. *P* values was found by using ANOVAand compared with significant (*P*≤0. 05). Non-significant values are *(P*≥0. 05).

Table 5: methanolic extract of *C. fistula* at 250mg/ml by at different pH.

Bacterial Strains	**Methanolic Extract of *C. fistula* at 250mg/ml by treating different pH**					**Control group**	***P* Value**
	3 pH	**5 pH**	**7pH**	**9 pH**	**11pH**		
Bacillus cereus	0.9±0.2	0.7±0.1	1.5±0.2	1.5±0.2	0.9±0.2	0±00	.001*
Bacillus subtilis	1.2±0.1	1.0±0.1	1.1±0.1	1.5±0.2	0.6±0.2	0±00	.000*
Escherichia coli	0.6±0.0	1.0±0.0	0.6±0.0	1.4±0.1	0.8±0.4	0±00	.002*
Staphylococcus aureus	1.2±0.2	1.8±0.4	2.4±0.2	2.8±0.4	1.5±0.2	0±00	.000*

Mean ± SD (n=3). Values present in rows are variables. *P* values was found by using ANOVAand compared with significant (*P*≤0. 05). Non-significant values are *(P*≥0. 05).

Conclusion: Based on findings, it can be concluded that *C. fistula* is an efficient source of herbal drugs thatcan be

used as supplement for a variety of diseases and that *C. fistula* leaves extract has antimicrobial activity against Gram positive bacteria *E.coli* and Gram negative *B. substilis* and *Staph. aureus*. Their methanolic extracts are extremely efficient and beneficial in the fight against bacteria. This study strengthens the fact of using herbal options instead of antibiotics which are associated with the high risk of health problems and development of anti-bacterial resistance in the pathogenic stains.

Conflicts of Interest: The author/s declare no conflicts of interest regarding the publication of this paper.

REFERENCES

Aslam, M. S. and Ahmad, M. S.Worldwide importance of medicinal plants: current and historical perspectives. Advances in Biology and Medicine, Vol 2, issue 2016, pp: 909, 2016.

Bhalodia, N. R. and Shukla, V. J. Antibacterial and antifungal activities fromleaf extracts of Cassia fistula l.: An ethnomedicinal plant. Journal of advanced pharmaceutical technology and research, Vol 2, issue 2, pp: 104, 2011.

Dutta, A. K. and Madharia, P. In- vitro Evaluation of Antibacterial Activity of Cassia fistula against different Gram- positive and Gram-negative Bacteria. Biomedical and Pharmacology Journal, Vol 5, issue 1, pp: 185, 2012.

Hamad, G. M., Darwish, A. M., Abu- Serie, M. M. and El Sohaimy, S. A. Antimicrobial, antioxidant and anti-inflammatory characteristics of combination (Cassia fistula and Ocimumbasilicum) extract as natural preservative to control & prevent food contamination. Journal of Food and Nutrition Research, Vol 5, issue 10, pp: 771-780, 2017.

Hassan, R. B. A. Medicinal plants (importance and uses). Pharmaceut. Pharmaceutica Analitica Acta, Vo 3, issue 10, pp: 2153-2435, 2012.

Mwangi,R. W., Macharia, J. M., Wagara, I. N. and Bence, R. L.Themedicinal properties of Cassia fistula L: A review. Biomedicine and Pharmacotherapy, pp: 144, 2021.

Nagpal, M. A., Nagpal, N., Rahar, S.,Shah, G., Swami, G. and Kapoor, R. Phytochemical investigation of methanolic extract of Cassia fistula leaves. Pharmacognosy Journal, Vol 3, issue 26, pp: 61-69, 2011.

Oladeji, O. The characteristics and roles of medicinal plants: some important medicinal plants in Nigeria. Natural Products Indian Journal, Vol 12, issue 3, pp: 102, 2016.

Seyyednejad, S. M., Motamedi, H., Vafei, M., and Bakhtiari, A. The antibacterial activity of Cassia fistula organic extracts. Jundishapur Journal of Microbiology, Vol 7, issue 1, pp: 2016.

Shaikh, S. A., Sidiqui, A., Naz, S. and Ismat, S.Detection of Food Poisoning (Bacillus cereus) Pathogen in Cooked and Refrigerated Rice Samples. Biological Sciences-PJSIR, Vol 62, issue 3, pp: 172-177, 2019.

Sofowora, A., Ogunbodede, E. and Onayade, A.The role and place of medicinal plants in the strategies for disease prevention. African Journal of traditional, complementary and alternative medicines, Vol 10, issue 5, pp: 210-229, 2013.

Ugboko, H. U., Nwinyi, O. C., Oranusi, S. U., Fatoki, T. H. and Omonhinmin, C. A. Antimicrobial importance of medicinal plants in Nigeria. The Scientific World Journal,Vol 2020, pp:1-10, 2020.

Yadav, R. N. S. and Agarwala, M. Phytochemical analysis of some medicinal plants. Journal of Phytology, Vol 3, issue 12, pp: 10-14, 2011.

Yuan, H., Ma, Q., Ye, L. and Piao, G. The traditional medicine and modern medicine from natural products. Molecules, Vol 21, issue 5, pp: 559, 2016.