International Journal of Phytomedicine 7 (2015) 18-22
http://www.arjournals.org/index.php/ijpm/index

Original Research Article

ISSN: 0975-0185

Antibacterial activity of herbal preparation –Agnijith

Karthiha N[1] , Gayathri Devi S[1]

***Corresponding author:**

Karthiha N

[1]Department of Biochemistry, Biotechnology and Bioinformatics
Avinashilingam Institute for Home Science and Higher Education for Women
Coimbatore 641 043, Tamil Nadu

Abstract

"Agnijith" a herbal preparation was evaluated against fifteen different microorganisms at different concentrations 120mg, 130mg, 150mg, 170mg and 190mg/100mg. Chloromphenicol was used as control. The *in vitro* antibacterial activity was performed by agar well diffusion method and minimum inhibitory concentration (MIC) method. "Agnijith" was found to be more potent in inhibiting the microorganisms such as Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas flourescence, Proteus mirabilis and Shigella dysentriae, whereas no inhibition zone was found against Micrococcus luteus, Streptococcus pyrogens, Enterococcus faecalis, Proteus vulgaris and Salmonella typhimurium by well diffusion method. The zone of inhibition ranges from 12mm to 25mm. Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus *aureus, Pseudomonas aeruginosa, Pseudomonas flourescence, Shigella dysentriae* were found to be sensitive at various concentrations by minimum inhibitory concentration method. The results clearly demonstrates that the "Agnijith" to be an effective antibacterial agent and could be used to treat diseases caused by microorganisms.

Keywords: antibacterial activity, herbal preparation, Agnijith, microorganisms, well diffusion method, MIC method.

Introduction

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs. Despite controversies, pharmacists are coming to terms with natural medicine being an important aspect of health delivery. The plant kingdom still held many species of plants containing substances of medicinal value which were yet to be discovered [4]. Medicinal Plants and their formulations are enormously used for treating a range of illness in ethnic medical practices as well as traditional system of medicine in India [7]. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs in developed countries about 80 per cent of plants are used in traditional medicine. For these reasons, herbal medicines and medicinal plants are not only meeting treatment needs in developing countries, but also getting popular in developed World. [6,8]. Fruits and vegetables are an important component of healthy diet. Some fruits like bananas offer great medicinal benefits. Bananas can be used to fight intestinal disorders; they can be used to promote healing. This fruit is also used as a treatment for burns and wounds. Banana leaves can be used as a cool compress for burns or wounds [5].

Curcuma longa Linn. belonging to family of Zingiberaceae, commonly known as turmeric and haldi in Hindi. *Curcuma longa* has been reported to possess antibacterial, antifungal and anti inflammatory activities. The parts used are rhizomes and it contains curcumin. *Curcuma longa* also contains protein, fats, vitamins all of which have an important role in wound healing and regeneration. Turmeric has been used for treating wounds in the rat.

The *Pandanus tectorious* is also known as fragrant screw pine. It functions as a pain reliever, mostly for headaches and pain caused by the arthritis. It can also be used as antiseptic and antibacterial, which makes it ideal for healing wounds. [1]

The objectives of the study is to evaluate the antibacterial activity of the herbal preparation "Agnijith" against fifteen different bacterial strains by well diffusion method and minimum inhibitory concentration method (MIC).

Materials and Methods

Collection of Sample

The Sample "Agnijith" which is claimed to heal the wound and burns is a combination of *Musa paradisica*- 4litre, *Pandanus tectorious*-100gms, *Curcuma longa* -25gms and Coconut oil-1Litre. The sample was obtained from Arya Vaidya Pharmacy, Ramanathapuram, Coimbatore and stored at room temperature until use.

Preparation of the Sample

The "Agnijith" herbal sample obtained from Arya Vaidya Pharmacy was in a semi solid form, was then heated over bunsen burner prior to use to make it to liquid form.

Collection of microorganisms

The bacterial strains used for the study were clinically isolated and obtained from the stock culture of Department of Biotechnology of PSGIMS Coimbatore. The pure isolates were inoculated on double strength agar slant and stored at 4ºC. The bacterial strains used were *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Pseudomonas fluorescence*, *Klebsiella sp*, *Shigella dysentriae*, *Micrococcus luteus*, *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*, *Streptococcus pyrogens*, *Proteus vulgaris* and *Salmonella sp*.

Assay of antibacterial activity using well diffusion Method

The antibacterial activity of the herbal preparation was tested against the microorganisms using the agar well diffusion inhibition test. The 24 hr broth culture of each of the test organisms were aseptically introduced and evenly spread using bent sterile nutrient agar plates. Three wells of about 6mm diameter were aseptically punched on each agar plate using a sterile cork borer, allowing at least 30mm between adjacent wells and between peripheral wells at the edge of the Petridis (Fixed volumes 120µl, 160µl, 180µl, 200µl and 230µl containing 120mg, 130mg, 150mg, 170mg and 190mg of the herbal preparation was then introduced into the wells. A control well was also filled with 100mg of chloromphenicol. The plates were then incubated at 37ºC for 24 hours and then screened for zone of inhibition.

Minimum Inhibitory Concentration Method (MIC) [3]

The minimum inhibitory concentration was defined as the lowest concentration of the sample that did not show any growth of the tested microorganism. In the study, 100µl of nutrient broth was taken in the wells of a 96 well diffusion. The 100µl of sample was mixed with100µl of sample diluents in the well making a twofold dilution then 100µl of this dilution was transferred and mixed with 100µl of the diluents in the second row making a 4:1 dilution. This proceeds consecutively down the plate making two fold dilution in each well. After the dilution of the sample are completed, an aliquot of test organism was added to all wells and the microtitre plates were incubated overnight. If the sample concentration was sufficient to kill the organism, no growth appeared and the wells will be clear. At the point there was insufficient sample to kill the organism and the well will be cloudy, indicating growth. That is the minimum inhibitory concentration (MIC) of the sample against that specific organism.

Result and Discussion

Antibacterial activity by well diffusion method[3]

The antibacterial activity of "Agnijith" was evaluated against fifteen different microorganisms by well diffusion method (Table 1 and Figure1). The herbal preparation "Agnijith" was found to be active at the concentration of 120mg, 130mg and 150mg and exhibited the inhibition zone of 20mm, 22mm and 25mm respectively against *Escherichia coli*. At the concentration of 150mg, 170mg, 190mg, *Proteus mirabilis* exhibited the inhibition zones of 12mm,14mm,15mm respectively. *Klebsiella pneumoniae* was found to be sensitive and exhibited the inhibition zone of 13mm,15mm and 21mm at the concentration of 150mg, 170mg, 190mg. The herbal preparation was found to be active and exhibited the inhibition zone of 12mm,14mm and 16mm against *Pseudomonas fluorescence* at the concentration of 150mg, 170mg and 190mg. *Klebsiella sp.,* exhibited the inhibition zone of 14mm and 15mm at 130mg and 150mg respectively. *Shigella dysentriae* at the concentration of 150mg, 170mg, 190mg exhibited the inhibition zone of 10mm,12mm and 16mm. *Bacillus subtilis* was found to be sensitive at the concentration of 120mg, 130mg, 150mg, 170mg, 190mg of the herbal preparation and produced a inhibition zone of 10mm,12mm,15mm,17mm, and 20mm respectively. "Agnijith" was also more effective on *Staphylococcus aureus* and exhibited the inhibition zone of 13mm,15mm,18mm, and 20mm at the concentrations of 130mg, 150mg, 170mg, and 190mg. "Agnijith" was found to be active at the concentrations of 130mg, 150mg, and 170mg exhibited the inhibition zone of 12mm, 14mm, 15mm *against Pseudomonas aeruginosa*. The remaining organisms *Micrococcus luteus, Enterococcus faecalis, Salmonella typhimurium, Streptococcus pyrogens, Proteus vulgaris, Salmonella sp.,* they did not show detectable inhibition against the herbal preparation.

The results revealed that the herbal preparation was found to be more potent in inhibiting the microorganisms such as Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Pseudomonas fluorescence, Bacillus subtilis, Shigella dysentriae, Escherichia coli and Proteus mirabilis. Hence it can be used as potent healing agent against the bacterial infections. Since no inhibition zone was found against Salmonella typhimurium, Enterococcus faecalis and Micrococcus luteus, it can be said that "Agnijith" was not effective against these organisms. Minimum Inhibitory Concentration Method (MIC)

The antibacterial activity of herbal preparation Agnijith was tested by Minimum inhibitory concentration method (TableII). At the

concentration of 120,130 and 150mg/100µl, the herbal preparation showed inhibition against the organisms *Escherichia coli* and *Klebsiella pneumoniae* whereas 130and 150mg/µl of Agnijith exhibited inhibition for the growth of *Klebsiella sp.*, Cultures *of Bacillus subtilis* were found to be sensitive at the concentration of 130,150 and 170mg/100µl. *Staphylococcus aureus* was sensitive at a concentration of 130mg, 150mg and170mg/100µl of the sample. The sensitivity of *Pseudomonas aeruginosa* was observed at the concentration of 140, 150 and 170mg/100µl. The growth of *Pseudomonas flouresence* was inhibited at a concentration of 150mg and170mg/100µl. *Shigella dysentriae* was found to be sensitive at the concentration of 150 and170mg/100µl. Agnijth was found to be less effective in inhibiting the growth of *Micrococcus luteus*, *Streptococcus pyrogens*, *Enterococcus faecalis*, *Salmonella typhimurium*, *Proteus vulgaris* and *Salmonella sp.*,

Conclusion

The antimicrobial potency of plants is believed to be due to tannins, saponins, phenolic compounds, essential oils and flavonoids [9]. Turmeric acts as a cholagogue, stimulating bile production, thus increases the digestion of fats and eliminating toxins from the liver. Wound healing and detoxifying properties of turmeric curcumin have also received considerable attention[10]. The ethyl acetate extract of *Curcuma longa* has demonstrated an excellent antibacterial activity. Hence from the present study, it can be concluded that the "Agnijith" herbal preparation could be used as a potent agent for diseases caused by microorganisms.

Table I Antibacterial Activity of Herbal Preparation "Agnijith" in terms of Zone of Inhibition against test organisms by well diffusion method

S.No	Bacterial Strains	Concentration of Agnijith in (mg)					Control
		120	130	150	170	190	
1.	*Escherichia coli*	20	22	25	--	--	27
2.	*Proteus mirabilis*	--	--	12	14	15	32
3.	*Klebsiella pneumoniae*	13	15	21	--	--	23
4.	*Pseudomonas fluorescence*	--	--	12	14	16	20
5.	*Klebsiella sp.,*	--	14	15	--	--	17
6.	*Shigella dysentriae*	--	--	10	12	16	30
7.	*Micrococcus luteus*	--	--	--	--	--	25
8.	*Bacillus subtilis*	10	12	15	17	20	27
9.	*Enterococcus faecalis*	--	--	--	--	--	15
10.	*Staphylococcus aureus*	--	13	15	18	20	24
11.	*Salmonella typhimurium*	--	--	--	--	--	13
12.	*Pseudomonas aeurginosa*	--	12	14	15	--	17
13.	*Streptococcus pyrogens*	--	--	--	--	--	18
14.	*Proteuus vulgaris*	--	--	--	--	--	17
15.	*Salmonella sp.,*	--	--	--	--	--	21

Zone of inhibition is expressed in mm, Control = 100mg Chloramphenicol

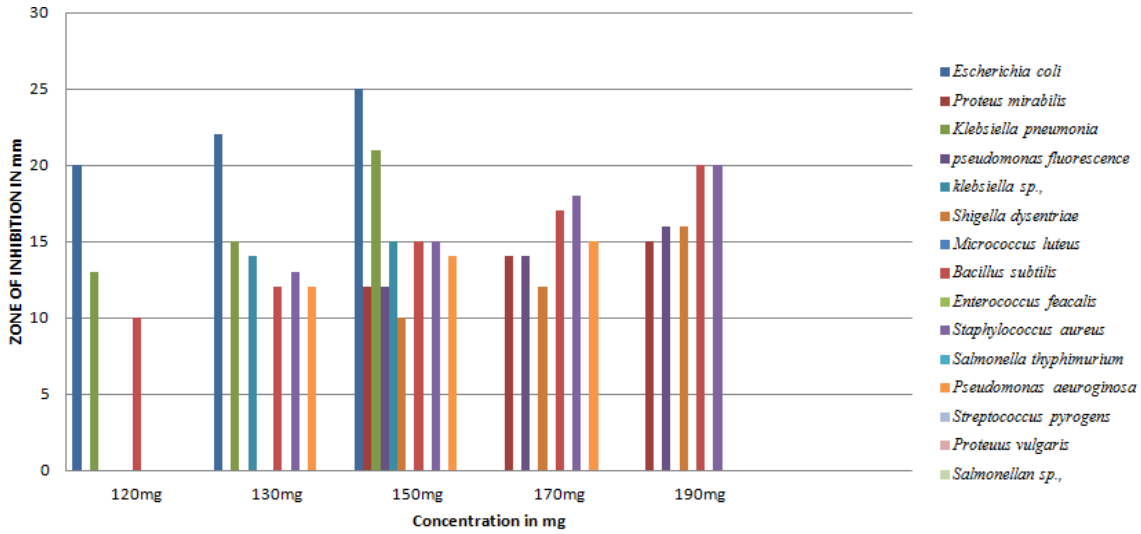

Figure I Antibacterial activity of Herbal Preparation "Agnijith" against test organisms by

well diffusion method Plate I

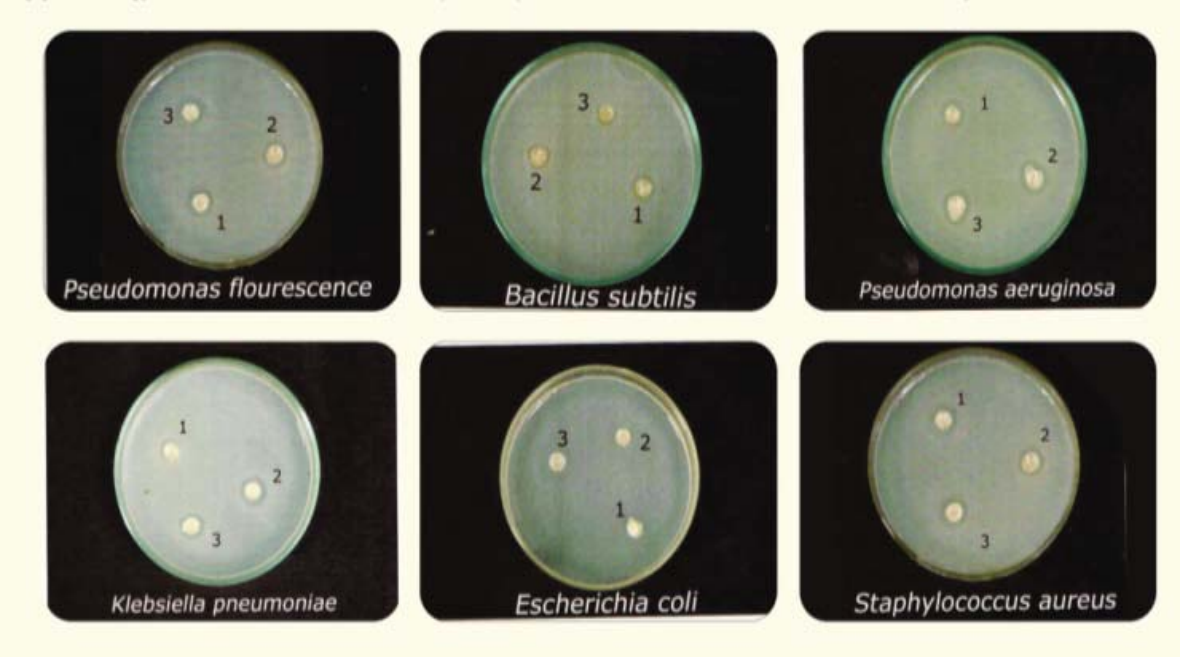

Table II Antibacterial activity of herbal preparation "Agnijith" by minimum inhibitory concentration method

S No.	Bacterial Strains	Concentration of Agnijth mg/100µl			
		120mg	130mg	150mg	170mg
1	*Escherichia coli*	+	+	+	-
2	*Proteus mirabilis*	-	-	+	+
3	*Klebsiella pneumoniae*	+	+	+	-
4	*Pseudomonas fluorescence*	-	-	+	+
5	*Klebsiella sp.,*	-	+	+	-
6	*Shigella dysentriae*	-	-	+	+
7	*Micrococcus luteus*	-	+	+	-
8	*Bacillus subtilis*	+	+	+	+
9	*Enterococcus faecalis*	-	-	+	+
10	*Staphylococcus aureus*	-	+	+	+
11	*Salmonella typhimurium*	-	+	-	-
12	*Pseudomonas aeruginosa*	-	+	+	+
13	*Streptococcus pyrogens*	-	-	-	-
14	*Proteus vulgaris*	+	-	-	-
15	*Salmonella sp.,*	+	+	-	-

+ indicates inhibition- indicates no inhibition

References

[1]. Gulzar A, Manju P and Anita Singh. Review and Research. Wound Healing potential of Some Medicinal Plants. J. of Pharmaceutical Sciences 2011;9(1) : article 026.

[2]. National Committee for Clinical Laboratory (NCCLS). Standards Methods for dilution, antimicrobial susceptibility test for bacteria that grow aerobically-Second Edition; Approved Standard, NCCLS document M7-A2. Villanova.Pa; NCCLS(1990),.

[3]. National Committee for Clinical Laboratory Standards (NCCLS). Performance Standard for Antimicrobial

susceptibility testing; Ninth Informational supplement. NCCLS (1999). 21.

[4]. Nwankwa LV, Amaechi N. Preliminary phytochemical screening and antimicrobial potentials of *Anacardium occidentales*. J. of research in antimicrobials 2013a; 1(2): 068-074.

[5]. Sambath kumar KP, Debjit B, Duraivel S and Umadevi MJ. of Pharmacognosy and Phytochemistry. 2012; 1(3); ISSN 2278-4136.

[6]. Shaahank M, Khosla KK, Cathrin M and Debjit B. Preliminary phyotochemical studies of *Kalanchoe pinnata* (Lann) pers. J. of Medicinal Plant Studies 2013; 1 (2); 19-23.

[7]. Shashank M, Ajay KJ, Majo J, Cathrin M and Debjit B. Analgesic and Anti- Inflammatory Activity of *Kalanchoe pinnata* (Lann) Pers. J. of Medicinal Plant Studies 2013; 1(2): 24-28

[8]. Vinod B, Devikirani P, Sanjay R, Ahul M. Science Research Report 2013; 3(1): 33-37.

[9]. Aboaba O, Efuwape BM, Antibacterial properties of some Nigerian species. Bio Res Comm. 2001;13:183–188.

[10]. Joe B, M. Vijaykumar and B.R. Lokesh. Biological properties of curcumin-cellular and molecular mechanisms of action. Food Sci. Nutr 2004; 44: 97-111.