WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 7, Issue 4, 1085-1094. Research Article ISSN 2277 – 7105

PHYTOCHEMICAL ANALYSIS AND ANTIMICROBIAL ACTIVITY OF *PERGULARIA DAEMIA* (FORSK) CHIOV, AGAINST MULTIDRUG RESISTANCE HUMAN PATHOGENIC BACTERIA

Madhuri M. Deshmukh* and Navnath G. Kashid

Department of Botany, Vasant Mahavidyalaya Kaij Dist: Beed (MS), India.

Article Received on
27 December 2017,

Revised on 17 Jan. 2018,
Accepted on 07 Feb. 2018

DOI: 10.20959/wjpr20184-11075

***Corresponding Author**
Madhuri M. Deshmukh
Department of Botany,
Vasant Mahavidyalaya Kaij
Dist: Beed (MS), India.

ABSTRACT

The increasing number of multidrug resistant bacteria to conventional antibiotics has become a serious problem in recent years. So there is alarming need to investigate new antibiotics and alternative products to solve this problem. This research aim is to search potent herbal drugs from *Pergularia daemia* extracted in different solvent system considering their polarity and non polarity nature. *Pergularia daemia* stem part extracted in different solvents, Methanol, Ethanol, Chloroform, Petroleum Ether, Ethyl Acetate and Aqueous solution. The technique used for extraction is of Soxhlet Apparatus and Rotary evaporator. Effective extracts obtain tested against some pathogenic bacteria, *Escherichia coli, (ATCC-25922), staphylococcus aureus (25923ATCC) Klebsiella pneumonia, Salmonella typhi (734 MTCC).* Among all tested extracts aqueous extract proved most powerful antibacterial activity compare to standard antibiotics amoxicillin for these bacteria, aqueous followed by ethyl acetate. Methanol and Ethanol extract showed less antibacterial effect. Chloroform and Petroleum Ether extract does not showed any activity. Antibacterial activity of solvent extracts was tested by Disc Diffusion and Agar Well Diffusion method. This research also highlights on the existing information particularly on the phytochemistry alkaloid, phenolic compound, steroids, flavonoids, which may provide incentive for proper evaluation of the plant as a medicinal agent.

KEYWORDS: *Pergularia daemia,* Antimicrobial activity, Human Pathogen, *E.coli, S. aureus, Kleb. Pneumoniae, S. typhi.* secondary metabolites.

INTRODUCTION

In Ayurveda the medicinal values of plants are well documented and revealed in the literature since ancient times. The compounds obtained from plants are rich in phytochemicals such as alkaloid, steroid, phenolic compound, tannin, protein and glycosides. Research in medicinal plants has gained a renewed focus recently. The prime reason is that synthetic drugs which used against certain diseases are effective but these are come with number of side effects that leads to serious complications. Plant produces some biomolecules which show antimicrobial activity. The increasing incidence of drug resistence pathogens has drawn attention of the pharmaceutical and scientific communities towards studies on the potential antimicrobial activity of plant derived substances. **(Sasmita Panigrahi and Sujata Mahapatra, 2016).** The plant synthesized a variety of compounds, including carotenoids, flavonoids, cinnamic acid, other polyphenolic compounds that prevent oxidation of the susceptible substrate and act as a natural antioxidant**.(Nagat M.*et al*. 2016).** Natural products either as pure compounds or as standardized plant extracts provide unlimited opportunities for new drugs. The future development of pharmacognistic analysis of herbal drugs is largely dependent upon reliable methodologies for correct identification, standardization and quality assurance of herbal drugs. **(Ranjita Saryam *et al.* 2012 and Shazia Sultana *et al* 2011).** The present study was aimed to investigate the antimicrobial activity of *pergularia daemia* against some multidrug gram positive and gram negative bacteria.

Botanical Description

Pergularia daemia is a perennial twining herb foul-smelling; stem bears milky juice and covered with small hairy structure of 1mm; leaves are thin, ovate or heart shaped with soft hair. Flowers born in axillary, double white corona at the base of stamina column, long peduncle, umbellate or corymbose clusters tinged with purple; Fruits paired with follicles 5.8 cm long and 1cm in diameter, refluxed, beak long, covered with soft spinous outgrowth and release many seeds with long white hair. Seeds are densely velvety on both sides. The entire plant constituents are used as a medicine (K.Karthishwaran and S. Mirunalini,2010).

Taxonomic Description:-
Kingdom : Plantae
Subkingdom: Tracheobionta
Super division: Spermatophyta
Division : Magnoliophyta
Class: Magnoliopsida
Sub class :-Asteridae
Order:- Gentianales
Family:- Asclepiadaceae
Genus:- *Pergularia*
Species:-*daemia*

Fig. I. *Pergularia daemia.*

Plant Phytochemistry: A number of phytochemical studies have demonstrated the presence of several chemical compounds. The leaves of *P. daemia* are contain flavonoids, alkaloids,terpenoids, tannins,steroids and carbohydrates (**Karthishwaran *et al.*, 2010)**.

Pharmacological Activity: Anti-inflammatory Activity: Pharmacological studies proved that *P. daemia* extract showed synergistic effect in treatment of various diseases. Ethanol extract and its butanol fraction exhibited significant anti-inflammatory activity when compared with standard drug aspirin **(Hukkeri et al., 2001).**

Anticancer Activity: It was also found that α-amyrin exhibited anticancer activity in low potency **(Khorombi et al. 2006).**

Antibacterial Activity: The promising antibacterial activity was observed in ethyl acetate and ethanol extracts of *P. daemia* against *S. aaureus, P. aeruginosa, A. hydrophila, E. coli and S. typhi*. (Senthilkumar et al.,2005). (Ignacimuthu et al., 2009) also isolated a new bioactive compound, 6-(4,7-dihydroxy-heptyl) quinine, a novel agent which is proved to be responsible for antibacterial activity.

Escherchia coli: *Escherichia coli* is a type of gram negative bacteria that normally lives in our intestine. Some pathogenic strain cause diarrhea if you eat contaminated food or drink fouled water. One especially bad strain, O157: of H7, causes abdominal cramps, vomiting, and bloody diarrhea. It is the leading cause of acute kidney failure in children.

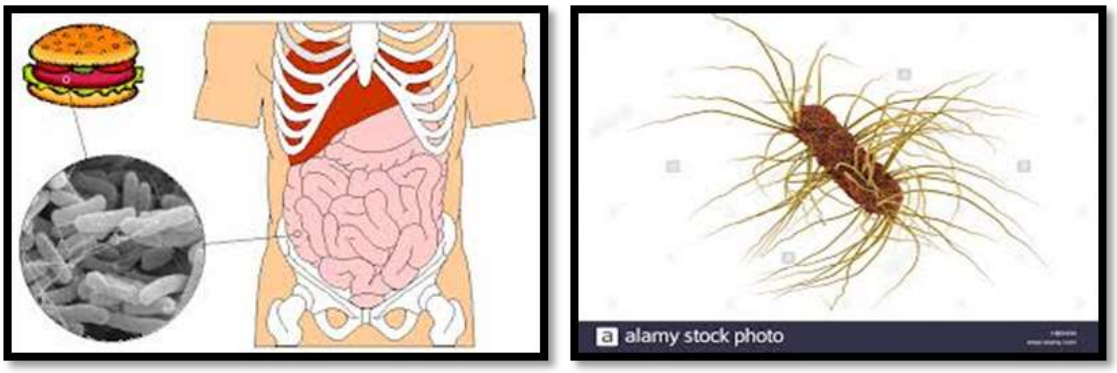


Fig. II: Intestinal infection caused by *Escherichia coli.*

Staphylococcus aureus: *S. aureus* is the most dangerous of all the many common staphylococcal bacteria. It is gram positive bacteria. It causes skin infection. They look like pimples or boils. They may red, swollen and painful and release pus. Some *step* bacteria are resistant to certain antibiotics, making infection harder to treat.

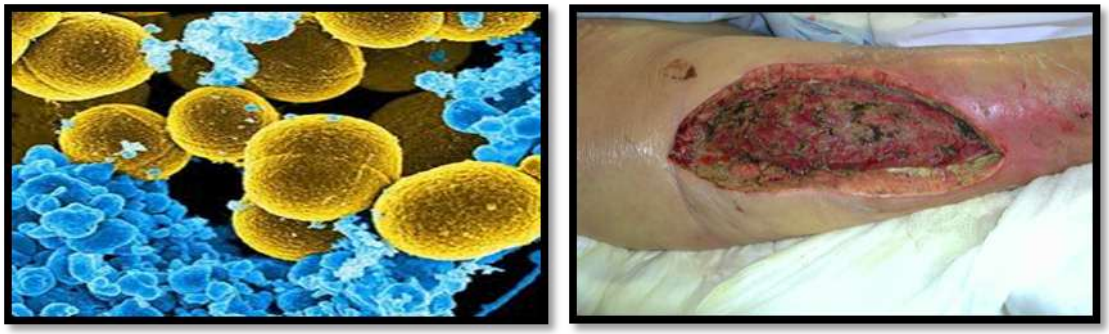


Fig. III: Infection caused by S. aureus.

Klebsiella pneumoniae: *Klebsiella pneumoniae* is nonmotile, gramnegative bacteria.It can cause pneumonia, bloodstream infection and meningitis. It is resistant to multiple antibiotics. Antibiotic therapy should be implemented for at least fourteen days.

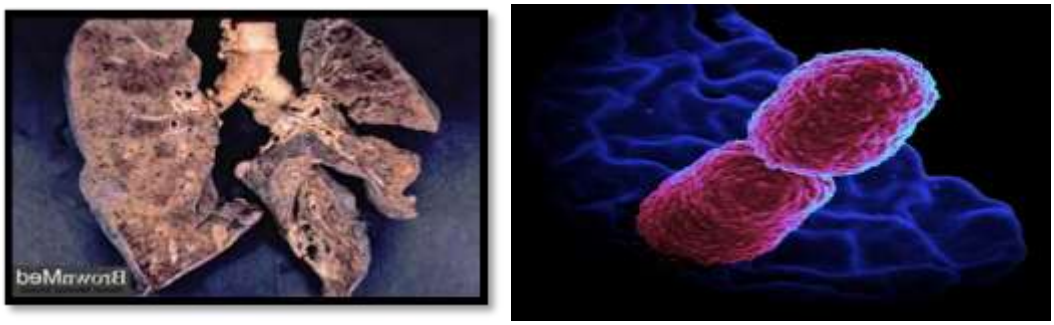


Fig. IV: Infection caused by *Kleb. Pneumoniae.*

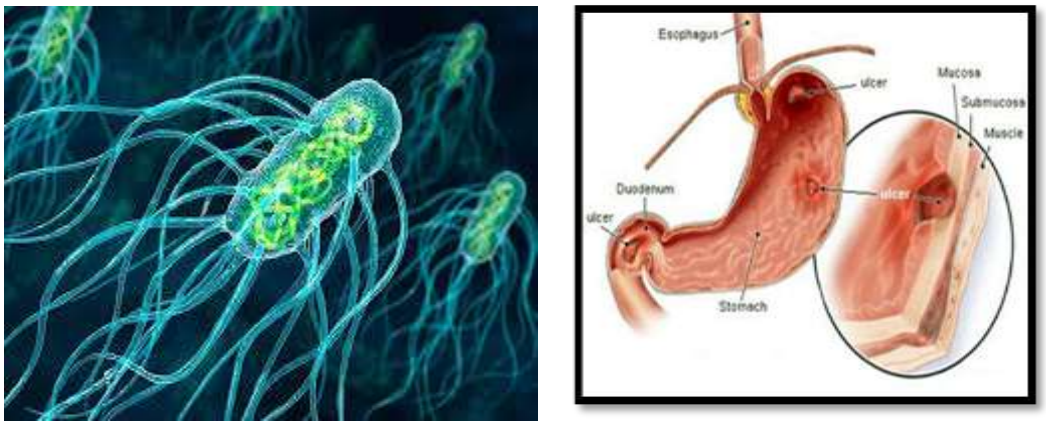


Fig. V: Infection caused by *S. typhi.*

Salmonella typhi: *S. typhi* is gram- negative bacteria. It is rod shaped and causes salmonellosis, a diarrheal illness in human. S. typhi infection is major cause of bacterial infections in the United States and India. According to the Centers for Disease Control and Prevention (CDC), it affects around one million people every year.

MATERIAL AND METHOD

Collection of Plant Material: The plant stem of *P. daemia* were obtained from local area of Aurangabad (MS), and authenticated with Accession number 641, by taxonomist Dr. Dhabe Arvind, Head Department of Botany Dr. Babasaheb Ambedkar Marathwada University Aurangabad. It get collected and washed with tap water followed by wipe with alcohol. Plant material kept under shade dry for about ten days. The *P. daemia* stem coarse powder obtained by using mortar and pestle. It passed through sieve 80mm; to obtain uniform size of particles. The powdered form of stem is stored in air tight glass jar for further use.

Bacterial Cultures

Gram Positive and Gram negative bacterial cultures *Staphylococcus aureus(25923ATCC), Klebsiella pneumonia, Escherichia coli (ATCC-25922), Salmonella typhi(734 MTCC),* are

used as test organisms. All the test strains were maintained on nutrient agar and were sub cultured once in every two weeks. The culture obtained from Clinical Microbiology Department Government Hospital Aurangabad.

Aqueous extraction: 200 gm of dried powdered plant stem was soaked in 1000ml of distilled water for 3-4 days at room temperature in dark condition. Then the water extract was filtered through filtered through filter paper and the filtrate was used for the phytochemical analysis.

Soxhlet extraction: The effective extraction technique of soxhelt used for extraction of biochemicals. Each weighed sample filled separately in 500ml soxhlet assembly, by adding respective solvent in the ratio of 1:5.(200gm :1000ml). The filtrate was concentrated to dryness under reduced pressure at 40º C using a rotary evaporator and stored at 4ºC for further use. Each extracts was resuspended in the respective solvent and used for the qualitative and quantitative analysis of phytochemicals. For the antimicrobial testing the extracts were reconstitute to a specific concentration in dimethyl sulphoxide (DMSO) **(Vijaya Packirisamy and Vijayalakshmi Krishna Moorthy, 2014).**

Antibacterial assay

Antibacterial activity of the plant extract was determined using a Kirby- Bauer disk diffusion method and Agar Well Diffusion Method. Briefly, 100 µl of the test bacteria were grown in 25ml of fresh nutrient agar media until they reached a count of approximately 108 cells of bacteria. And then 100µl of microbial suspension was spread on to the Nutrient Agar Plates and sterile wells were made with the help of sterile cork borer. The Methanol, Ethanol, Chloroform, Pet. Ether and Aqueous extract of *Pergularia daemia* (100µg/ml) were added to the two wells in aseptic conditions. The above Plates were incubated at 37°C for 24 hours, and then the diameters of the inhibition zones were measured.

Each antibacterial assay was performed in triplicate & Mean Values were reported. Standard antibiotic, Amoxicillin (100µg/well) served as positive controls for antimicrobial activity

Table 1: Phytochemical tests of *P. daemia.*

Sr. No	Phytochemicals	Aqueous extract	Ethyl acetate extract	Ethanol extract	Methanol extract	Chloroform Extract	Petroleum ether extract
1.	Tannins	+++	+	++	++	+	-
2.	Saponins	-	-	-	-	-	-
3.	Flavonoids	+++	+++	+	+	-	-
4.	Quinones	++	+	++	+	-	+
5.	Betacyanins	++	+	_	+ +	+	-
6.	Anthocyanins	-	-	-	-	-	-
7.	Steroids	-	-	-	-	-	-
8.	Alkaloids	+++	+ +	+	+	+	-
9.	Glycosides	+	-	+	+	-	-
10.	Terpenoids	+	+	++	+	-	+
11.	Cardiac glycosides	-	-	-	-	-	-
12.	Phenols	+++	+	++	+	-	-

Whereas:+++:Strongly present, ++:Mildly present , + Present and – Absent.

RESULT AND DISCUSSION

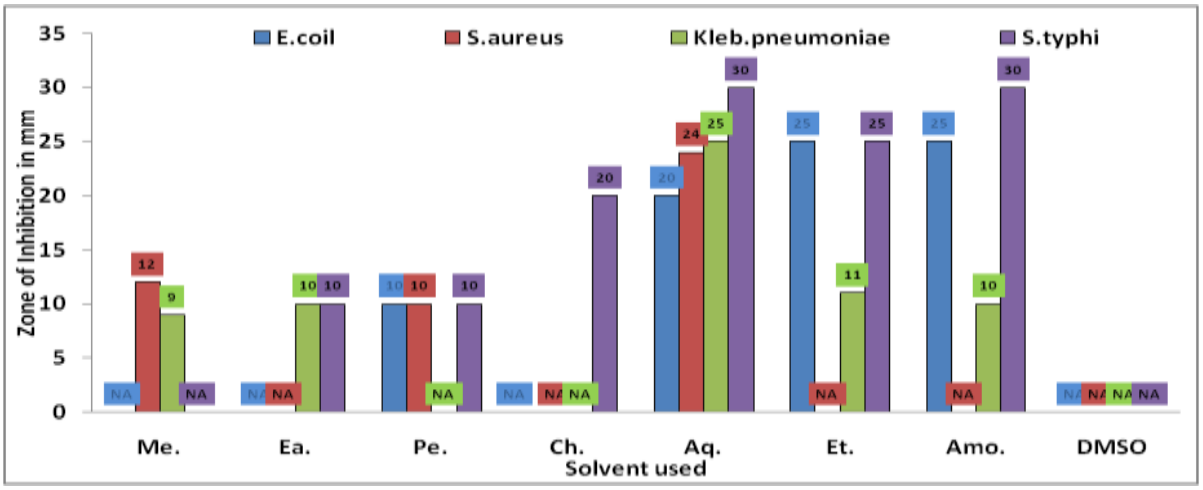
The preliminary phytochemical analysis of different solvents extracts flavonoids, tannins, phenolic compounds, Quinones, steroids, terpenoids and saponins as illustrated in table.1. The overall results focuses that plant extracts may be potent bacteriostatic/ bactericidal agents against bacterial strains.

The results from this investigation indicates that the *P. daemia* aqueous and ethyl acetate extracts offer significant potential for the development of novel antibacterial therapies and the treatments of several disease caused by microorganisms.

Table 2: Name of solvent extracts and their antibacterial activity against selected human pathogens.

Sr. no	Solvent used	Zone of inhibition in(mm)			
		E. coli	***S.aureus***	***Kleb.pneumoniae***	***S. typhi***
1.	**Me.**	NA	**12**	**09**	**NA**
2.	**Ea.**	NA	NA	**10**	**10**
3.	**Pe.**	**10**	**10**	**NA**	**10**
4.	**Ch.**	NA	NA	NA	**20**
5.	**Aq.**	**20**	**24**	**25**	**30**
6	**Et.**	**25**	NA	**11**	**25**
7.	**Amo.**	**25**	NA	**10**	**30**
8.	**DMSO**	**NA**	**NA**	**NA**	**NA**

Me-Methanol, Ea-Eathonal, Pe. Pet. Ether, Ch-Chloroform, Aq-Aqueous,Et-Ethyl acetate Amo-Amoxicillin, DMSO-dimethyl Sulphoxide.



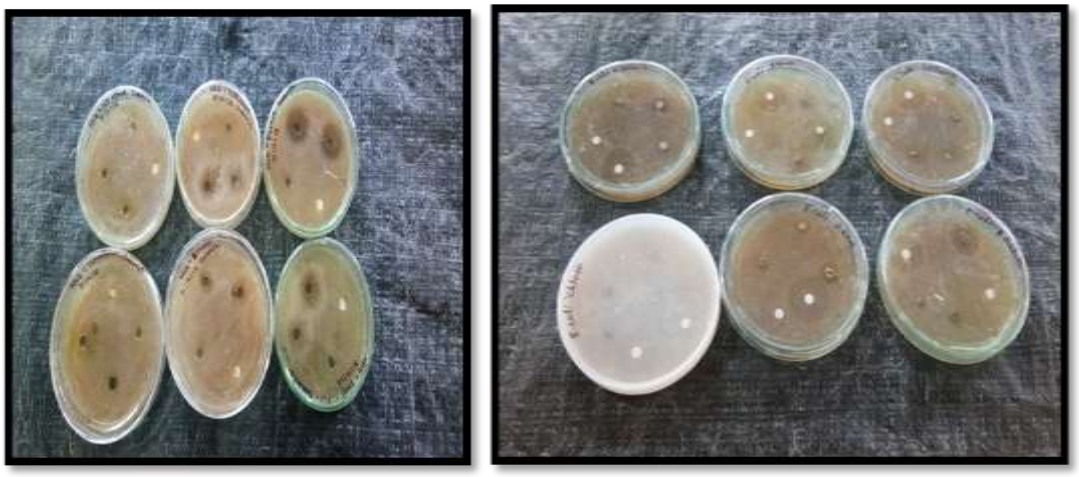


Fig. VI: Antibacterial Activity of *p. daemia Fig.VII.* Antibacterial Activity *of P.daemia* against *E. coli. against s. aureus.*

Fig. VIII: Antibacterial Activity of P.daemia Fig. IX. Antibacterial Activity of P. daemia against Kleb. Pneumonia. against S. Typhi.

CONCLUSION

From the present experiment the aqueous extracts of *Pergularia daemia* has antibacterial activity due to the presence of active constituents. However further research will be needed for identification of the bioactive compounds of the plant which are responsible for the pharmacological action against the disease causing microorganisms.

ACKNOWLEDGEMENT

We express our gratitude to Dr. Wakte, Head of Department of Chemical Technology for support to carry initial part of our research. Authors are thankful to Prof. Dr. Dhabe Head Dept. of Botany BAMU Aurangabad, for authentication of *Pergularia daemia.*

REFERENCES

1. Hukkeri, V. I., M. B. Patil, S.S. Jabalpure and A. Ali, 2001. Antiinflammatory Activity of various extracts of *Pergularia extensa N.E.Br(Asclepiadaceae). Indian J. Pharmaceutical Sci.*, 63: 429-431.l.4(8)
2. Ignacimuthu, S., M. Pavunraj, V. Duraipandiyan, N. Raja and C. Muthu, 2009. Antibacterial Activity of a novel quinine from the leaves of pergularia daemia (Forsk.), a traditional medicinal plant. Asian J. Tradi. Med., 4: 36-40.
3. K.Karthishwaran and S.Mirunalini, 2010. Therapeutic Potential of Pergularia daemia (Forsk.): The Ayurvedic Wonder.International Journal of Pharmacology, 6(6): 836-843. 50-256.
4. Karthishwaran, S.mirunalini, G. Dhamodharan, M. Krishnaveni and V. arulmozhi, 2010 Phytochemical Investigation of Methanolic Extract of the leaves of Pergularia daemia, J. Biological science,10; 242-246.
5. Khandelwal K.R., Practical Phamacognosy, 2002. Techniques and experiments. Pune; Nirali Prakashan.
6. Khorombi, T.E., G. Fouche and F. R. Van Heerden., 2006. Phytochemical Investigation and the anticancer properties of *Pergularia daemia* and *Phylicapaniculata.* CSIR. http://researchspace.csir.co.za/dspace/bitstream/10204/2804/1/khorombi-2006-D.
7. Nagat M., Ehmadi Barka, Reena Lawrence, Mariya Saani,. 2016. Phytochemical screening, Antioxidant and Antibacterial Activity of active compounds from *Hemidesmus indicus*, 8(2).
8. Ranjita Saryam, Chandrabhan Seniya, Shagufta Khan., 2012. Physico-chemical and Preliminary Phytochemical Screening of *Hemidesmus indicus.* Jocpr., 4(11): 4695-4697.

9. Sasmita Panigrahi and Sujata Mahapatra., 2016 Evaluation of Antibacterial Activity of *Pongamia pinnata* L., *curcurma longa* L. *and Mentha arvenis* L. Against *Staphylococcus aureus*, 9(02): 205-212.
10. Senthilkumar, M., P. Gurumoorthi and K. Janardhanan, 2005. Antibacterial Potential of some plants used by tribals in Maruthamalai hills, Tamil Nadu. Nat. Prod. Radiance, 4: 27-34.
11. Shazia Sultana, Mirajab Khan, Mushtaq Ahmad, Asghari Banu, Muhammad Zafar and Zabta Khan Shinwari. Pak, 2011; J. Bot., 43: 141-150, Special issue.