Plant Archives Vol. 17 No. 2, 2017 pp.1665-1668 *ISSN 0972-5210*

A STUDY OF PHYTOCONSTITUENTS AND ANTIMICROBIAL POTENTIAL OF DIFFERENT LEAF EXTRACTS OF *PAEONIA EMODI* WALL.

Meena* and N.S. Bisht

Department of Botany, H.N.B. Garhwal University, Campus Pauri, Pauri Garhwal, (Uttarakhand) India.

Abstract

The present study is focused to evaluate the antimicrobial efficacy of *Paeonia emodi* wall. (Paeonaceae) against *Escherichia coli*, *Baccillus subtilis* and *Staphylococcus aureus*. *Paeonia emodi.* The leaves and rhizomes are used as a tonic to cure backbone ache, backache, dropsy and epilepsy and also used as emetic, cathartic, blood purifier and colic while the seeds are purgative. Present investigation is an attempt to reveal the antibacterial activity of the aqueous, methanol and ethanol extracts of *Paeonia emodi* leaves using the well diffusion method and measuring zone of inhibition. The highest zone of inhibition was observed in methanol extract against *B. subtilis* (22.00 mm) followed by *Staphylococcus aureus* (18.00 mm) and *E. coli* (14.96 mm). And in case of ethanol extract the highest zone of inhibition was observed in against *B. subtilis* (21.00 mm) followed by *E.coli* (17.16mm) and *Staphylococcus aureus* (16.80 mm). A comparision among three extracts, the methanol extract showed highest while the aqueous extract exhibited lowest zone of inhibition. The results of the extracts were also compared with the standard antibiotics.

Key words: *Paeonia emodi* Wall., Antimicrobial, Well Diffusion, Zone of Inhibition.

Introduction

The use of higher plants and their preparation to treat infectious and non-infectious disease is an age old practice and was the only method available in the past. Though the use of natural sources like plant material for curing diverse forms of ailments leads to human civilization, the scientific analysis of different natural sources for their possible medicinal potency is comparatively recent origin. The emergence and spread of antibiotic resistance of microorganisms triggered this type of plant investigations. (Cowan, 1999) Hence, the plant kingdom is being screened for newer and effective chemotherapeutic agents. Higher plants can serve both as potential antimicrobial crude drugs as well as a source of new anti-infective agents. (Rios, 2005).

Phytomedicine can be used for the treatment of diseases as is done in case of Unani and Ayurvedic system of medicines (Barnes *et al.* 2002). This plant-based, traditional medicine system continues to play an essential role in health care, and play dual role in the development of new drugs: they may become the base for the development of a medicine, a natural blue print for the development of new drugs or; as phytomedicine to be used for the treatment of diseases (Khera, *et. al.* 2012).

Materials and methods

Plants were collected from the oak forest near Chandrapuri on way to Kedarnath (2100-3000m). Aqueous, methanol and ethanol extracts of the plant were prepared using standard techniques and the final extracts was 1g dry material/ ml. (Panthi and Chaudhari, 2006).

Glycosides, polyphenols, tannin, flavonoid, saponins, steroids terpenoid and alkaloids were analysed following standard methods. (Sofowra, 1993, Trease, and Evans 1989, Harborne, 1983).

Three species of bacteria, two gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and one gram-negative (*Escherichia coli*) were cultured in nutrient broth for 24 hours and the fresh inoculums were taken for the test and reconfirmed by gram staining and sub culturing in appropriate selective media. Three to four isolated colonies were inoculated in 2 ml nutrient broth and incubated till the growth in the broth was

Author for correspondence : E-mail : meenu19botany@gmail.com, nsbotany@gmail.com

equivalent with Mac-Farland standard (0.5%) as recommended by WHO at which the number of cells was assumed to be 1.5 × 108 cfu/ml.

The antibacterial activity was assessed by agar well diffusion method (Panthi and Chaudhry 2006). Different concentrations ranging from 10mg/ml to 30mg/ml of the working suspension/solution of different plant extracts were loaded in each well and same volume of extraction solvent for control was filled in the wells with the help of micropipette. The tests were performed three times and the zones of inhibition were measured for each extract using a ruler and the results were recorded.

Results and Discussion

Phytochemistry of *Paeonia emodi:*

The phytochemical analysis of aqueous, methanolic and ethanolic leaf extracts of *Paeonia emodi* showed the presence of glycosides, polyphenols, tannins, flavonoids, saponins, steroids, terpinoids and alkaloids. These all constituents are known to exhibit medicinal as well as physiological activities. All the three extracts shown variation in the concentration of these phytochemicals. Intense colour with +++ mark corresponding to higher quantity of phytochemicals. Higher quantity of glycosides were observed in the ethanolic extract while both methanolic and ethanolic extracts exhibited intense colour in case of polyphenols and tannins. Flavonoids were found in higher quantity in methanol extract only. Other phytochemicals were noticed in moderate quantity (table 1).

This plant possesses medicinal properties due to phytochemicals present in the plant as alkaloids have been reported to their analgesic antispasmodic and antibacterial properties. (Stray 1998, Okwu and Okwu, 2004). According to many reports glycosides are known to lower the blood pressure (Nyarko and Addy 1990). Various studies shown phenolic compound possess biological properties such as apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection and improvement of endothelial function, as well as inhibition of angiogenesis and cell proliferation and antimicrobial activities (Han and Shen 2007). The plant extracts were also revealed to contain saponins which are known to produce inhibitory effect on inflammation (Just *et al.*, 1998). Steroids have been reported to their antibacterial properties (Epand, *et al.,* 2007). The growth of many fungi, yeasts, bacteria and viruses can be inhibited by tannins (Chung *et al.,* 1998).

Zones of inhibition:

Among all the pathogens, all gram negative and positive bacteria were inhibited by all type of plant extracts. The plant extracts shown inhibitory action against *Escherichia coli, Bacillus subtilis* and *Staphylococcus aureus* (table 1, 2, 3 and 4). The zones of inhibition of the aqueous, methanolic and ethanolic leaf extracts and their different concentrations for *E. coli* ranged from 0 mm - 10.63±0.48 mm (aqueous extract), 5.25±0.6 mm - 14.96±0.05 mm (methanol extract) and 0 mm - 17.16±0.36 mm (ethanol extract). In case of *Bacillus subtilis* it ranged from 5.07±0.4 mm to 12.43±0.51 mm (aqueous extract), 6.33±0.25 mm-22.0±0.3 mm (methanol extract), 6.07±0.6- 21.06±0.5 mm (ethanol extract), while for *Staphylococcus aureus.* the range was from 3.63±0.55 mm-10.73±0.42mm, 6.06±0.2 mm-18.0±0.4 mm 7.37±0.63 mm-16.8±0.13 mm for aqueous, methanol and ethanol extract respectively which was comparable to standard antibiotics (erythromycin and tetracycline 30ig, table 5). The extract showed least activity against *E. coli*. with minimum zone of inhibition. Gram positive bacterial strains were found to be slightly more sensitive than Gram negative bacterial strains. It may be due to the absence of lipopolysachride layer in Gram positive bacteria that might function as a barrier to the phytocemical substances that are responsible for antibacterial activity (Martin, 1995; Brantner *et al*., 1996; Palombo and Semple, 2001; Tortora *et al*., 2001; Matu and van Staden, 2003).

The results of the present study on the antimicrobial potentials of *Paeonia emodi* against *Escherichia coli, Bacillus subtilis,* and *Staphylococcus aureus* have shown that the aqueous, methanolic, and ethanolic extracts of the plant inhibited the growth of the test isolates. Among the three extracts the methanolic extract shown greater ZOI followed by ethanol and aqueous extracts. There is an indication that the extracts possess substances that can inhibit the growth of some micro-organisms. However, the observed inhibitory effects were more as the antimicrobial potential of *Paeonia emodi* concentrations of the extracts increase from 10 mg/ml, to 30 mg/ml. *Escherichia coli, Bacillus subtilis.* and *Staphylococcus aureus* at concentrations of 30 mg/ml each exhibited more inhibition zones.

The presence of various active ingredients secondary plant metabolites as revealed by the phytochemical screening (table: 1) supports the resourcefulness of the plant extracts (Sofowora, 1993). Generally the methanol extract shows the highest activity against both bacterial and fungal isolates, which is due to the active ingredients of the plant parts and these are better extracted with methanol than other solvents. The methanol extracts contain alkaloids, and tannins which have antibacterial and antihelminthic properties (Saeedi *et al.,* 2008). These secondary metabolites exert antimicrobial activity through different mechanisms. Tannins have been found to form irreversible complexes with proline-rich proteins resulting in the inhibition of the cell protein synthesis. (Hagerman *et al.,* 1998).

The traditional healers use primarily water as the

Table 1: Variation of phytochemicals in different extracts of *P. emodi.*

S. No.	Phytochemicals	Aqueous extract	Methanol extract	Ethanol extract
1.	Glycosides	+	++	+++
2.	Polyphenol &Tanins	+	+++	+++
3.	Flavinoids	+	+++	++
4.	Saponines	+	+	+
5.	Steroids	+	++	+
6.	Terpenoids	+	+	++
7.	Alkaloids	+	++	++

+ (minimum), ++ (moderate), +++ (High)

Table 2: Zone of inhibition (ZOI) in different concentration of aqueous extract.

Test Micro organism	Aqueous leaf extract different concentration					
	10mg/ml (ZOI)mm		20mg/ml (ZOI)mm		30mg/ml (ZOI)mm	
Bacteria	Mean/	SD	Mean	SD	Mean	SD
E.coli	0	0	5.33	0.76	10.63	0.48
B.subtilis	5.07	0.40	6.54	0.56	12.43	0.51
S.aureous	3.63	0.55	8.17	0.6	10.73	0.42

Table 3: Zone of inhibition (ZOI) in different concentration of methanol extract.

Test Micro organism	Aqueous leaf extract different concentration					
	10mg/ml (ZOI)mm		20mg/ml (ZOI)mm		30mg/ml (ZOI)mm	
Bacteria	Mean/	SD	Mean	SD	Mean	SD
E.coli	5.25	0.6	7.66	0.57	14.96	0.05
B.subtilis	6.33	0.25	9.06	0.6	22.0	0.3
S.aureous	6.06	0.2	10.33	0.15	18.0	0.4

Table 4: Zone of inhibition (ZOI) in different concentration of ethanol extract.

Test Micro organism	Aqueous leaf extract different concentration					
	10mg/ml (ZOI)mm		20mg/ml (ZOI)mm		30mg/ml (ZOI)mm	
Bacteria	Mean/	SD	Mean	SD	Mean	SD
E.coli	0	0	12.0	0.09	17.16	0.36
B.subtilis	6.07	0.60	10.67	0.53	21.6	0.50
S.aureous	7.37	0.63	8.77	0.68	16.8	0.13

solvent but we found in this study that the plant extracts in alcohol (ethanol and methanol) provided more consistent antibacterial activity compared to those extracted by water. Successive extraction and isolation of botanical compounds from plant material is largely dependent on the type of solvent used in the extraction

Table 5: Zone of inhibition (ZOI) of Standard Antibiotics.

Test Micro organism	Standard Antibiotics (Positive Control)			
	Tetracycline (30µg) (ZOI)mm		Erythromycin (30µg) (ZOI)mm	
Bacteria	Mean/	SD	Mean	SD
E.coli	11.0	0.03	12.1	0.04
B.subtilis	16.07	0.05	13.4	0.06
S.aureous	13.37	0.07	13.2	0.03

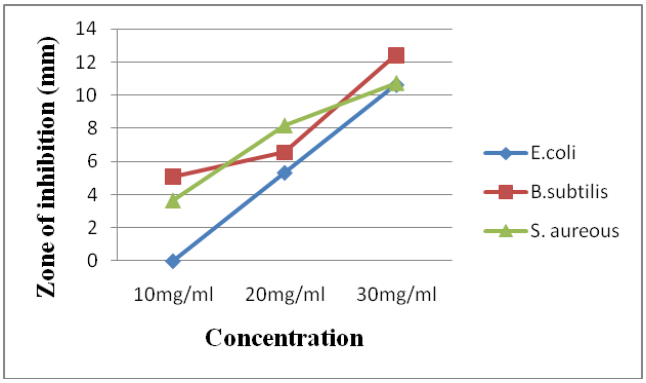

Fig. 1: Zone of inhibition of different concentration of aqueous extract.

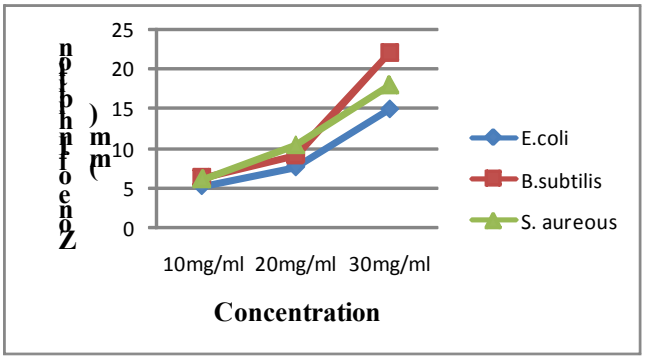

Fig. 2: Zone of inhibition of different concentration of methanol extract.

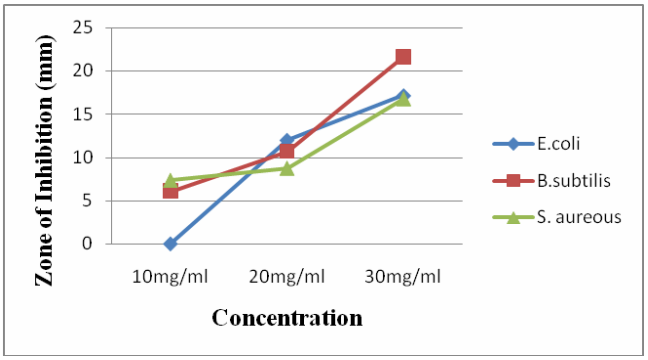

Fig. 3: Zone of inhibition of different concentration of ethanol extract.

procedure.

The high altitude grown Himalayan plant *Paeonia emodi* has not been investigated for its defined antimicrobial potential. Though it has been exploited for

Plate: A), B) and C) : Zone of inhibition: *E.coli, B. subtilis and S. aureous*

A) E.coli B) ***B. subtilis*** C) ***S. aureous***

its other uses all over the world but the indigenous plant part, especially rhizome needs to be tested adequately for its antimicrobial potential. This study highlights for the first time the ability of solvent extracts of the plant *Paeonia emodi* as antibacterial through *in vitro* assays against pathogens.

Acknowledgment

The authors acknowledge their profound gratitude to the Head, Department of Botany, H.N.B. Garhwal University Srinagar for providing the necessary lab facilities for the current research work. And also thankful to UGC non net fellowship for financials aids during the research work.

References

Barnes J., L.A. Anderson and J.D. Phillipson (2002). Herbal medicines. A guide for healthcare professionals, 2nd edn. Pharmaceutical, London, 468–476.

Brantner, A., Z. Males, S. Pepeljnjak and A. Antolic (1996). Antimicrobial activity of Paliurus spina-christi Mill (Christ's thorn). *J. Ethnopharmacol,* **52**: 119-122.

Chung, K.T., C. Wong, Y. Wei, Huang and Y. Lin (1998). Tannins and Human Health: A Review, *Critical Reviews In Food Science and Nutrition*, **38(6)**: 421-464.

Cowan,M.M.. Plant products as antimicrobial agents. *Clin Microbiol Rev.*; **12**: 564-582.

Epand, R.P. Savage, and R. Epand (2007). Bacterial lipid composition and the antimicrobial efficacy of cationic steroid compounds (Ceragenins), *Biochimica Et Biophysica Acta Biomembranes*, **1768(10)**: 2500-2509.

Hagerman, A.E. and I.G. Butler (1998). High molecular weight plant polyphenolics (Tannins) as a biological antioxidents. *J. Biol. Chem,* **256**: 4494-4497.

Han, X.T. Shen and H. Lou (2007). Dietary Polyphenols and Their Biological Significance, *IJMS*, **8(9)**: 950-988.

Harborne. I.B. (1973). Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn, Chapman and Hall, New York, pp. 88-185.

Just, M.J. M.C., R. Giner, M.J. Cuellar, S. Manez, A.R. Bilia and J.L. Rios (1998). Anti-Inflammatory Activity of Unusual Lupane Saponins from Bupleurum fruticescens, *Planta Med*, **64(5)**: 404-407.

Khera, N., Y. Thakur and A.Bhatia (2012). *Asian Journal of Plant Science and Research*, **2(5)**: 638-642.

Martin, G.J. (1995). Ethnobotany: A MethodsManual. London: Chapman and Hall.

Matu, E.N., J. van Staden (2003). Antibacterial and anti-inflammatory activities of some plants used for medicinal purposes in Kenya. *J. Ethnopharmacol,* **87**: 35-41.

Nyarko, A. and M. Addy (1990). Effect of aqueous extract ofAdenia cissampeloides on blood pressure and serum analytes of hypertensive patients, *Phytother. Res.*, **4(1)**: 25-28.

Okwu, D. and M. Okwu (2004). Chemical composition of Spondias mombin linn. plant parts. *Journal of Sustainable Agriculture Environment*, **6(2)**: 140-147.

Palombo, E,A. and S,J. Semple (2001). Antibacterial activity of traditional Australian medicinal plants. *J. Ethnopharmacol,* **77**: 151-157.

Panthi, M.P. and R.P. Chaudhari (2006). Antibacterial activity of some selected folklore medicinal plants from west Nepal. ***Scientific World*** **4**:16-21.

Rios, J.L. and M.C. Reico (2005). Medicinal plants and antimicrobial activity. *J. Ethnopharmacol,* **100**: 80-84.

Saeedi, M.K., Morteza-Semnani, M.R. Mahdavi and F. Rahimi, (2008). Antimicrobial studies on extracts of four species of *Stachys, Indian. J. Pharm Sci.,* **70**: 403-406.

Sofowora, A. (1993). Medicinal Plants and Traditional Medicine in Africa. John Wiley and Sons Limited, **2**: 96-106.

Stray, F. (1998). The Natural Guide to Medicinal herbs And Plants (pp. 12-16) (London: Tiger Books International.

Tortora, G.J., B.R. Funke and C.L. Case (2001). Microbiology: An Introduction. San Francisco: Benjamin Cummings.

Trease, G,E. and WC. Evans (1983). Textbook of Pharmacognosy. 12th edn. Balliese Tindall and Company Publisher,London. pp. 343-383.