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COMPARATIVE EVALUATION OF ANTBACTERIAL ACTIVITIES OF PUNICA GRANATUM LEAVES AND ACACIA LEUCOPHLOEA ROXB. LEAVES

Brijesh Sharma[1]*, Lal Ratnakar Singh [2], Geetika Gupta[3], Deen Dayal Singh[4], Ashok Kumar[5]

*[1]Associate Prof., Dept. of Pharmacy, IBMER, Mangalayatan University, Beswan, Aligarh, U.P.-202146, India.

[2]Principal, Dept. of Pharmacy, IBMER, Mangalayatan University, Beswan, Aligarh, U.P. -202146, India.

[3]Senior Research Fellow, Animal Health Division, Makhdoom, Farah, Mathura.

[4]Senior Research Fellow, Animal Health Division, Makhdoom, Farah, Mathura.

[5]Principal Scientist, Animal Health Division, Makhdoom, Farah, Mathura.

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***Corresponding Author**
Brijesh Sharma
Associate Prof., Dept. of Pharmacy, IBMER, Mangalayatan University, Beswan, Aligarh, U.P.-202146, India.

ABSTRACT

In the present investigation, the aqueous and methanolic leaf extracts of Punica granatum L. and Acacia leucophloea Roxb. were subjected to phytochemical screening using various qualitative tests. Their physical characteristics and antibacterial activity against Escherichia coli, Streptococcus and Pseudomonas aeuroginosa, was evaluated visually and by disc diffusion method, respectively. A comparison was made between antibacterial activity of both the above plants. The results revealed the presence of Phenols, tannins, alkaloids, flavonoids, saponins, glycosides, terpenoids, carbohydrates, proteins and lipids in P. granatum leaves and A. leucophloea leaves consisted of steroids in addition to the above constituents. The results further showed that both the extracts were effective against all the tested bacteria and antibacterial activity increased with an increase in concentration of extract. The maximum zone of inhibition (mm.) against E. coli, P. aeuroginosa and Streptococcus were detected to be 20, 14, 20 and 13, 18, 23 for methanolic and aqueous leaf extracts of P. granatum, respectively. Similarly, the maximum zone of inhibition (mm.) against E. coli, P. aeuroginosa and Streptococcus were found to be 13, 16, 17 and 17, 12, 22 for methanolic and aqueous leaf extracts of A. leucophloea,

respectively. Nature of solvent considerably affected their antibacterial activity. Overall, P. granatum was found to have greater antibacterial activity against all the tested bacteria. The results of study suggested that the leaf of both above plants can be used as effective antibacterial agents for treating bacterial infections, supported their use as Ayurvedic phytomedicine and their combination in the form of some suitable dosage forms for topical application could yield better results in case of skin infections.

KEYWORDS: Phytochemical, antibacterial, phytomedicine, zone of inhibition.

INTRODUCTION

The excessive use of commercially available antimicrobials caused the emergence of antimicrobial resistant pathogens that ultimately posed a threat to global public health (Khan and Hanee, 2011). Since developing and testing new antimicrobial a drug is expensive and all antibiotics with prolonged usage may have adverse effects on human health because they kill gut flora. Therefore, attention has now-a-days diverted towards natural products for treating diseases. The Indian subcontinent has a rich flora of various plants used in traditional medical treatments (Ballabh, and Chaurasia 2007). Plant extracts and compounds derived from plants are in use as drug from the ancient times (Kamal and Khan 2014). *Acacia leucophloea Roxb* also called *reonja*, is a moderate sized tree and it attains a height of about 20 to 30 ft and a girth of 2 to 3 ft (Kaul, 1963), belongs to the family *Fabaceae* under the subfamily Mimosoideae. Its largest continuous distribution is arid India through Sri Lanka, Bangladesh, Burma and much of Thailand (Nielsen, 1992). The chemical constituents found are n-Hexacosanol, beta- Amyrin, beta-Sitosterol and Tannin. Traditionally the bark is used as astringent, bitter, thermogenic, styptic, alexeteric, antihelmintic, vulnerary, demulcent, constipating, expectorant and antipyretic, vulnerary, demulcent, constipating, bronchitis, cough, vomiting, wounds, ulcers, diarrhoea, dysentery, internal and external haemorrhages, dental caries, oral ulcers, proctoptosis, stomatitis and intermittent fevers (Bhadoria and Gupta 1981, Gupta *et al.* 2012). *Punica granatum* L., commonly known as pomegranate, is a fruit bearing deciduous shrub or small tree, native to Asia and belongs to family Lathraceae (Altuner, 2011). This plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties (Dahham *et al.* 2010, Inabo *et al.* 2011, Moussa *et al.* 2011). It has been highlighted in many studies as having antimicrobial activity against a range of both Gram positive and negative bacteria (Navarro *et al.* 1996). Chemical constituents of the leaf extract of *P. granatum* are almost similar to those of the fruit or seed,

e.g., ellagic acid, tannins (punicalin and punicafolin), and flavones glycosides including luteol in and apigenin (Bisht *et al.* 2016). The present investigation was undertaken with the objective of comparing antibacterial activity in methanolic and aq. leaf extracts of A. leucophloea and P. granatum which in turn could help in the development of some suitable topical formulaton for treating various dermatological disorders.

MATERIALS AND METHODS

Materials: All the reagents, viz. beef extract, acid hydrolysate of casein, starch and agar used in the present study were of analytical grade and procured from Hi Media laboratory.

METHODS

Collection and Authentication of Plant Material

Fresh leaves of P. granatum and A. leucophloea were collected from the herbal garden maintained at C.I.R.G. Makhdoom, Farah, Mathura, U.P. They were authenticated and identified by Dr. Sanjay Kataria, Head of Dept., Botany, BSA degree college, Mathura.

Processing of raw plant material

Plant material after collection was shade dried. Dried plant material was coarsely grinded with the electric grinder and stored in air tight containers till further use.

Preparation of Crude Extracts

The shade dried powder of leaves was subjected to soxhlet extraction using water and methanol as solvents. The extracts were filtered individually using Whatmann filter paper no.

1, evaporated to dryness in rotary evaporator and the percent yields of both the extracts were determined. They were stored at -20 ^{0}C (Kasliwal and Quadri, 2016).

Determination of extraction yield: The yield (%, w/w) from the above dried extracts were calculated by given below formula:

Yield (%) = (W_1 x 100) / W_2

Where, W_1 is the weight of the extract obtained after drying of solvent and W_2 is the weight of the plant powder.

Evaluation of Characteristics of Extracts

The physical state, color, odor, taste and percent yield of the two leaves extracts were determined.

Antimicrobial Screening of Extracts By In Vitro Method

Microbial Strains

All the strains of microorganisms were obtained from C.I.R.G., Makhdoom, Farh, Mathura. Details of bacteria used for study are given in table 1.

Table 1: Details of Bacteria Used for Study.

S. No.	Name of bacteria	Strain
1.	Escherichia coli	Gram-negative bacteria
2.	Pseudomonas aeruginosa	Gram-negative bacteria
3.	*Streptococci*	Gram-positive bacteria

Evaluation of Antibacterial Activity by Disc Diffusion Method (Kumar et al. 2015):

Muller Hinton Agar (MH Agar) media was used for the investigation. The composition of media is given below:

Beef extract-	:	2.00 gm.
Acid hydrolysate of casein-	:	17.50 gm.
Starch-	:	1.5 gm.
Agar –	:	17.00 gm.
Final pH-	:	7.3 ± 0.2
Distilled water-	:	1000 ml

The prepared media was stored below 8 ^{0}C, protected from direct sunlight.

Initially, the stock cultures of bacteria were revived by inoculating in Brain Heart Infusion (BHI) broth media and grown at 37 ° C for 24 hrs. The plates of MH media were prepared. The 24 h old bacterial cultures (100µ, 104 CFU) were spread evenly on the plates. Extracts of plants to be tested were applied on sterile discs in specific concentration. The extracts were left for drying at room temperature for 20 minutes. After drying, the extracts along with discs were applied on MH media plates. All the plates were incubated at 37°C for 24 h and the diameter of Zone of Inhibition (ZOI) in mm. were noted (Kumar *et al.* 2013).

RESULT AND DISCUSSON

Preparation of Extracts

The leaves extracts of the plants under study, namely Punica granatum and Acacia leucophloea Roxb. were successfully prepared by soxhelt apparatus (hot method) followed by evaporation of solvent in rotary evaporator.

Extraction of Extracts

Characteristics of methanolic extracts of Punica granatum and Acacia leucophloea Roxb. Leaves

The physical state, color, odor, taste and percent yield of the two leaves extracts are given in table 2 and 3. Various reports are published on physical characteristics of crude extracts (Charde *et al.*2014). The percentage yield in the present investigation is almost similar to that of crude extract of C. carandus (29.54 %) by Sharma, 2005.

Table 2: Characteristics of Methanolic Leaves Extracts.

S. No.	Characteristics	Observations	
		Punica granatum L.	Ac Acacia leucophloea Roxb.
1.	Physical state	Solid	Semi-solid
2.	Color	Dark green	Brownish-black
3.	Odor	Characteristic	Pungent
4.	Taste	Bitter	Bitter
5.	Percent yield	29.26	28.57

Table 3: Characteristics of aq. Leaves Extracts

S. No.	Characteristics	Observations	
		Punica granatum L.	Ac Acacia leucophloea Roxb.
1.	Physical state	Solid	Semi-solid
2.	Color	Dark green	Brownish-black
3.	Odor	Characteristic	Pungent
4.	Taste	Bitter	Bitter
5.	Percent yield	28.67	27.85

Phytochemical screening of Punica granatum and Acacia leucophloea Roxb. Leaves extracts

The results of phytochemical analysis of the methanolic and aqueous leaves extracts of Punica granatum L. and Acacia leucophloea Roxb. are presented in table 4. Phenols, tannins, alkaloids, flavonoids, saponins, glycosides, terpenoids, carbohydrates, proteins and lipids were found to be present in P. granatum extract while A. leucophloea extract contained steroids also in addition to the above constituents. The results of phytochemical analysis are

similar to the previous published reports (Kumar *et al.* 2015, Prabha *et al.* 2015, Suneetha *et al.* 2016).

Table 4: Phytochemical screening of P. granatum L. and A. leucophloea Roxb. Leaves extracts.

S. No.	Test for Phytoconstituents	Leaves extracts			
		Punica granatum		Ac Acacia leucophloea Roxb.	
		Aqueous	Methanolic	Aq Aqueous	M Methanolic
1.	Phenols	+	-	+	-
2.	Tannins	+	+	+	+
3.	Steroids	-	-	+	+
4.	Alkaloids	+	+	+	+
5.	Glycosides	+	+	+	+
6.	Flavonoids	+	+	+	+
7.	Saponins	+	+	+	+
8.	Terpenoids	+	+	+	+
9.	Carbohydrates	+	+	+	+
10.	Proteins	+	+	+	-
11.	Lipids	+	+	+	+

(+ indicates the Presence of Phytocompound; − Indicates the absence of phytocompound).

In Vitro Testing of Extracts for Antibacterial Activity

Antibacterial activity of both extracts was determined by disc diffusion method. Zone of inhibitions (ZOI) were measured in mm. Both the methanolic and aq. extracts under study (Punica granatum and Acacia leucophloea Roxb.) leaves exhibited significant antibacterial activity against all the tested bacteria, namely E. coli, Pseudomonas aeuroginosa, and Streptococci. The results of antibacterial activity are shown in table 5 and 6. From the above table, it is clear that the antibacterial activity of both the extracts were concentration dependent, i.e., as the concentration increased, antibacterial activity also increased. The maximum zone of inhibition (mm.) against E. coli, P. aeuroginosa and Streptococci were detected to be 20, 14, 20 and 13, 18, 23 for methanolic and aqueous leaf extracts of P. granatum, respectively. Similarly, the maximum zone of inhibition (mm.) against E. coli, P. aeuroginosa and Streptococci were found to be 13, 16, 17 and 17, 12, 22 for methanolic and aqueous leaf extracts of A. leucophloea, respectively. Furthermore, table 5 shows that the methanolic extract of P. granatum had more antibacterial activity or greater ZOI than A. leucophloea Roxb. against E. coli while aq. extract of A. leucophloea Roxb. was found to have greater antibacterial activity than aq. extract of P. granatum against E. coli. The table 5

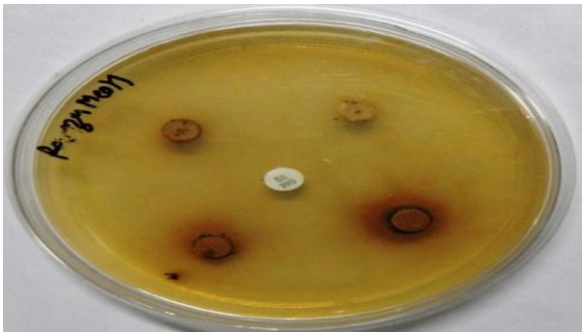
also revealed that methanolic extract of A. leucophloea was having greater antibacterial activity than methanolic extract of P. granatum against P. aeruginosa. Conversely, aq. extract of P. granatum leaves was found to be more effective antibacterial agent than aq. extract of A. leucophloea leaves (table 6). Thus, the nature of solvent considerably affected the antibacterial activity of extracts. Hegde *et al.*(2012) also reported similar ZOI of methanolic leaf extract against E. coli (13 mm.) and slightly less (8 mm.) than our ZOI (10 mm.) of aq. leaf extract. However, Rathinamoorthy *et al.*(2011) detected higher antibacterial activity of methanolic and aq. leaf extract of P. granatum against E. coli. This could be due to the variation in concentration of extract used. Tannins, alkaloids, saponins and flavonoids have been found to be active antibacterial agents (Kennedy and Withtman, 2011). Similar to our results, Dabur *et al.* (2007) has also reported low antibacterial activity of A. leucophloea bark. Rahman *et al.* (2014) and Lawrence *et al.* (2015) have reported antibacterial activity of A. nilotica and A. Arabica bark, respectively against E. coli, Streptococcus, P. aeurogiosa. Similar to our results, Kumar *et al.* (2015) also reported concentration-dependent increase in ZOI of P. granatum leaf against Streptococcus. Overall, P. granatum was found to have greater antibacterial activity against all the tested bacteria.

Table 5: Antibacterial Activity of Methanolic (MeOH) Leaf Extracts of P. granatum and A. Leucophloea.

Bacteria	**Plant Extract (Zone of inhibition in mm.)**							
	P. granatum (MeOH)				**A. leucophloea (MeOH)**			
Concentration (mg. / ml.)	500	250	125	62.5	500	250	125	62.5
E. coli	20	20	15	13	13	12	10	10
Streptococcus	20	15	11	10	17	15	10	10
P. aeurogiosa	14	13	12	11	16	14	13	10

Table 6: Antibacterial Activity of Aqueous (Aq.) Leaf Extracts of P. granatum and A. Leucophloea.

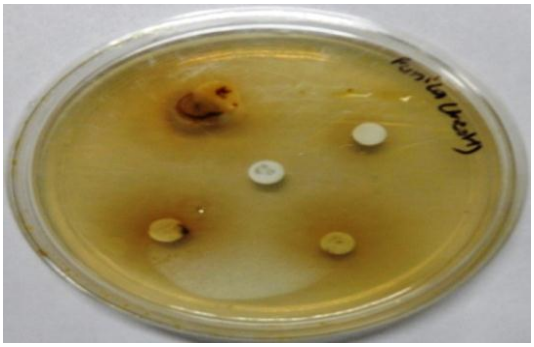
Bacteria	**Plant Extract (Zone of inhibition in mm.)**							
	P. granatum (Aq.)				**A. leucophloea (Aq.)**			
Concentration (mg. / ml.)	500	250	125	62.5	500	250	125	62.5
E. coli	13	11	10	10	17	15	13	10
Streptococcus	23	21	16	12	22	16	13	11
P. aeurogiosa	18	15	14	13	12	11	10	10



Methanolic Leaf Extract

Aqueous Leaf Extract

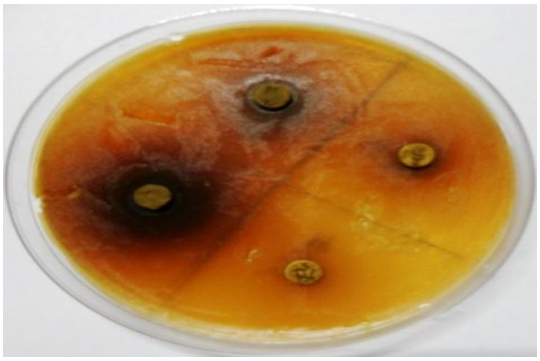
Figure 1: Antibacterial Activity of A. leucophloea Extracts Against Streptococci.



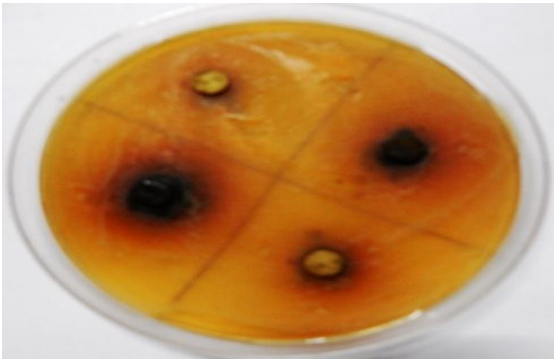
Methanolic Leaf Extract

Aqueous Leaf Extract

Figure 2: Antibacterial Activity of P. granatum Extracts Against Streptococci.

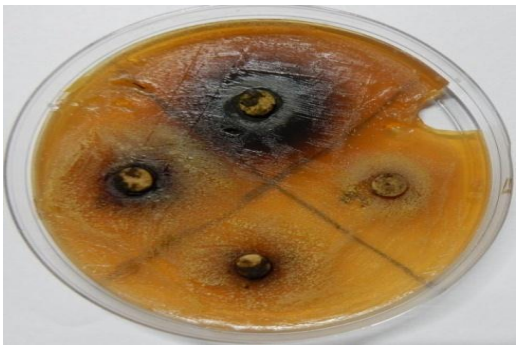


Methanolic Leaf Extract

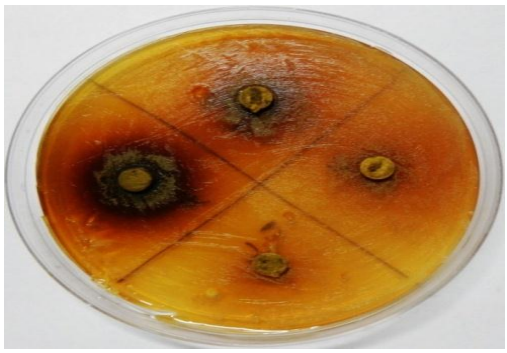


Aqueous Leaf Extract

Figure 3: Antibacterial Activity of A. leucophloea Extracts Against E. coli.

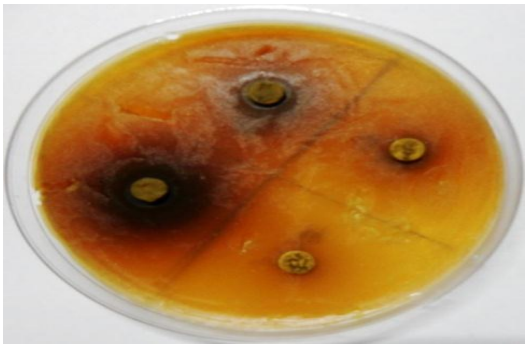


Methanolic Leaf Extract

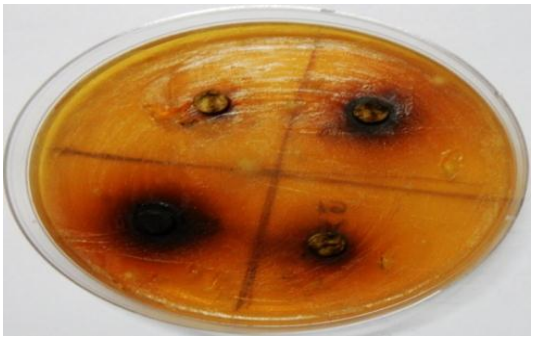


Aqueous Leaf Extract

Figure 4: Antibacterial Activity of P. granatum Extracts Against E. coli.

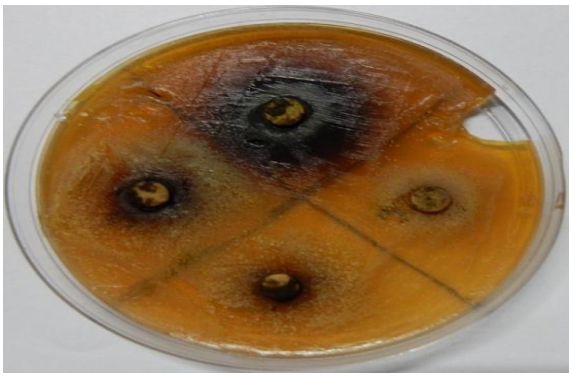


Methanolic Leaf Extract

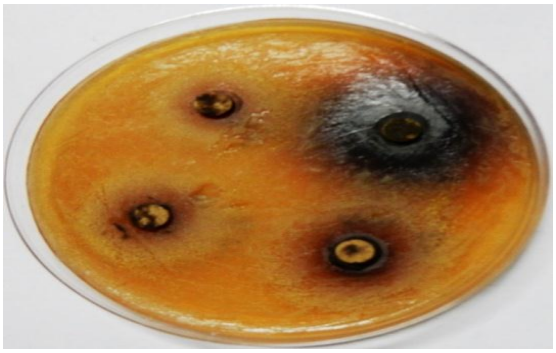


Aqueous Leaf Extract

Figure 5: Antibacterial Activity of A. leucophloea Extracts Against P. aeuroginosa.



Methanolic Leaf Extract



Aqueous Leaf Extract

Figure 6: Antibacterial Activity of P. granatum Extracts Against P. aeuroginosa.

CONCLUSION

The present investigation was conducted with the purpose of comparing antibacterial activity in the methanolic and aqueous leaf extracts of Punica granatum L. and Acacia leucophloea Roxb. against E. coli, P. aeuroginosa, and Streptococci. The results indicated that both the extracts had remarkable antibacterial activity against all the tested bacteria but overall P. granatum was found to have higher zone of inhibition. Furthermore, the nature of solvent affected the antibacterial activity of extracts to a great extent. For some strains, methanol was better solvent while for others water was proved to be more effective solvent for determination of antibacterial activity. Furthermore, antibacterial activity was concentration-dependent, i.e. as concentration increases, activity also increased. Thus, it can be inferred from the results that a judicious combination of above crude drugs in appropriate ratio could be used for the development of suitable topical formulation like antimicrobial gel, cream etc. which in turn could have applications in a variety of dermatological disorders.

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