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Research Article

ANTIMICROBIAL, ANTIOXIDANT AND HEMOLYTIC ACTIVITIES OF BLUMEA LACERA

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ABSTRACT

The present study focuses on the phytochemical analysis, antioxidant activity, RBC haemolysis and antimicrobial activity of *Blumea lacera* leaf. The phytochemical screening of aqueous and methanolic leaf extracts revealed the presence of various secondary metabolites such as alkaloids, phenolic compounds, tannins, flavonoids, glycosides and saponins. The study revealed that the leaf extracts of *Blumea lacera* showed antibacterial activity against various organisms and also showed significant RBC haemolysis activity. The study shows that the methanolic extracts significant antioxidant activities in a concentration dependant manner. The plant contains potential antibacterial components that may be useful for evolution of pharmaceutical for the therapy of ailments.

INTRODUCTION

Many secondary metabolites of plant chemicals are derived biosynthetically from plant primary metabolites. The secondary metabolites can be classified into several groups on the basis of their chemical classes. Medicinal plants are responsible for their efficacy, was often used to identify plants for treating diseases (Parekh and Chanda, 2007). Plants contain many active compounds such as alkaloids, steroids, tannins, glycosides, volatile oils, fixed oils, resins, phenols and flavonoids which are deposited in their specific parts such as leaves, flowers, bark, seeds, fruits, root, etc (Kumar *et.al*.,2006).

Medicinal plants are rich in wide variety of secondary metabolites such as terpenoids, alkaloids, tannins, flavonoids, etc, which have antimicrobial properties. The plant *Blumea lacera* belongs to family Asteraceae. It is commonly called as Janglimulli, Kakaronda, Siyalmutra, and Susksampatra is a camphoraceous smelling, tall stem, corymbosely branched herb. It is a perennial plant, with obovate, deeply serrated leaves and yellow groundsel-like flowers, the whole plant being thickly clothed with long silky hairs. It is found growing wildly in wastelands, roadside areas and also found in drying ponds along drains and river margins. *Blumea* is described by Ayurveda experts as hot, pungent and bitter; antipyretic; good for bronchitis, diseases of the blood, fevers, thirst and burning sensations. The root kept in the mouth is said to cure disease of the mouth. In the Konkan region of India, the plant is used to drive away fleas and other insects. *Blumea lacera* is used in folk medicine for the treatment of cough, bronchitis, and dysentery, wound healing (Salisu *et.al*., 2015).

Haemolytic activity of any compounds is an indicator of general cytotoxicity towards normal healthy cells. Usually, saponins (a group of phytochemical) present in the plants showed haemolytic activity by creating changes in the erythrocyte membrane. In vitro haemolytic assay by spectroscopic method provides an easy and effective method for the quantitative measurement of hemolysis. This method provides the evaluation of the effect of different concentrations of biomolecules on the human erythrocytes (Bhaskara Rao *et.al*., 2011). In this study, *Blumea lacera* was screened for the haemolytic activity.

Antioxidant assay, the purple chromogenic radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) is reduced by antioxidant/reducing compounds in the sample to the corresponding pale yellow hydrazine. This involves the odd electron of nitrogen atom in DPPH getting reduced by receiving a hydrogen atom from antioxidants. The procedure involves measurement of decrease in absorbance of DPPH at

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its absorption maxima of 517 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution (Farooq *et.al.,* 2010). DPPH assay is a rapid, simple, in-expensive and widely used spectrophotometric method to measure the ability of compounds to act as free radical scavengers or hydrogen donors. DPPH assay is considered a valid accurate, easy and economic method to evaluate radical scavenging activity of antioxidants, since the radical compound is stable. Also, the results are highly reproducible and comparable to other free radical scavenging methods (Mathur *et. al*., 2011).

MATERIALS AND METHODS

Plant material collection

The plant material *Blumea lacera* leaves were bought from Karjat village in January 2017. The leaves were dried in hot air oven at 60°C-65°C for 12hrs and blended into powder, this powder of *Blumea lacera* were used for aqueous and methanol extraction and phytochemical analysis. The dried plant materials were ground to fine powder and stored in air tight dark bottles at room temperature. 10 g of each plant materials were extracted by mixing with distilled water (100 ml), 60% methanol (100 ml) and 95% ethanol (100 ml) in a waterbath at 40°C for 30 min. Extracts were filtered through Whatman No.1 paper filter and collected. Ethanol and methanol extracts were concentrated to dryness in a soxhlet apparatus at 60°C for 30 min and aqueous extracts were freeze dried. All extracts were stored at 4°C until further analysis (Salisu *et.al*., 2015).

Phytochemicals screening

The freshly crude extract was tested for the presence of active principles such as:-
Test for Tannins, Glycosides, Phenol, Flavonoids and Alkoloids using different solvents and subjected to the quantitative phytochemical analysis (Tiwari *et.al.,* 2011).

RBC haemolysis assay

In vitro haemolytic activity was performed by spectrophotometer method (Bhaskara Rao *et.al*., 2011).
Materials used for haemolysis assay was human blood obtained from pathology Lab., Vasai (E).

Preparation of HRBC suspension

Test: Different concentrations (0.2, 0.6, 1.0, 5.0, 10, 15, 20 mg/ml) of crude extract were made by diluting it in PBS. 975ul of this was incubated with 25ul of erythrocyte suspension for ten minutes at room temperature. After incubation the tubes were centrifuged at 3000rpm for 5mins and the supernatant was decanted in fresh eppendorf tube for further use. Its absorbance was determined spectrophotometrically at 560nm. Phosphate buffer saline and distilled water were used as minimal and maximal haemolytic controls. Each experiment was performed in triplicates at each concentration.

Calculation

% Haemolysis= 100× [(test-color blank-negative control) / (positive control-negative control)]
The H50, the concentration at which 50% haemolysis was obtained, was determined from the concentration-response curves.

Antioxidant Activity

Method used for antioxidant activity was DPPH free radical scavenging assay.

DPPH Free Radical Scavenging Assay

To determine antioxidant activity 2,2-diphenyl-1-picryl-hydrezyl (DPPH) was used as free radical. 100 µM concentration of DPPH was used in methanol. Serial dilutions were made to check the IC50. In 96-well micro plate total volume was 100 µl which was consisting of 90 µl of DPPH solution and 10 µl of the test solution. The contents were mixed and incubated for 30 minutes at 37°C. To determine the absorbance at 517 nm synergy HT BioTek USA micro plate reader was used. Ascorbic acid was used as standard antioxidant (Salisu *et.al*., 2015; Mishra *et.al*.,2015). All readings were taken in triplicate. Ez-fit-5, Perrella Scientific Inc., Amherst, USA, software was used to calculate the IC50. Decrease in absorbance indicated increased radical scavenging activity which was determined by the following formula:

$$\%\ \text{radical Scavenging} = \frac{\text{Absorbance of Control} - (\text{Absorbance of Test} - \text{Absorbance of Blank}) * 100}{\text{Absorbance of Control}}$$

Where absorbance of control = total radical activity without inhibitor and absorbance of test = activity in the presence of test compounds.

Determination of antimicrobial activity

Strains and media

The human pathogenic micro-organisms - *Staphylococcus aureus* (2602)*, Escherichia coli (*ATCC10536)*, Sterptococcus pyogenes*(2608)*, Klebsiella pneumoniae* (Clinical isolates), *Salmonella typhi* (ATCC 23564) and *Candida albicans* (Clinical isolates) used to test the antimicrobial activity of the plant extracts. All bacteria strains were grown in Nutrient broth at 37°C.

Antimicrobial activity of plant extracts

Evaluation of the antibacterial activity of the crude ethanolic, methanolic hot and cold and aqueous extracts of *B. lacera* parts was determined by the agar well diffusion method (Silvia *et. al*., 2013; Shoba *et. al*., 2014). Inoculum of the bacterial strains was spread using sterile spreaders into 90 mm petri dishes with Mueller–Hinton agar. Wells of 6 mm were cut with help of sterile cork borer and filled with 50µL of 30 mg/ml of solvent extracts. Empty wells were used as negative control. The Petri dishes were incubated for 3 h at room temperature for complete diffusion of the samples (Das *et al*., 2010).

RESULT AND DISCUSSION

Results obtained in the present study revealed that the four extracts of plant *Blumea lacera* posses potential antibacterial activity against *S. aureus, E. coli, S. pyogens, K. pneumonia* and *S. typhi.* When tested by the Agar cup method, the hot aqueous extract showed highest antibacterial activity against all organisms, more than 12mm. Cold aqueous extract showed significant activity against all four organisms and hot methanol showed lowest activity against *E. coli, S. pyogens*, and *K.*

pneumonia whereas, cold methanol did not showed any activity against all five organisms.

Table 1 Microorganisms with strain number and suitable media at suitable incubation temperature

Sr.No.	Name of the cultures	NCIM	Growth media	Incubation Tem.
1	*Escherichia coli*	ATCC10536	Nutrient agar	37ºC
2	*Salmonella typhi*	ATCC 23564	Nutrient agar	37ºC
3	*Staphylococcus aureus*	2602	Nutrient agar	37ºC
4	*Streptococcus pyogenes*	2608	Nutrient agar	37ºC
5	*Candida albicans*	Clinical isolates	Sabouraud agar	Room temp.
6	*Klebsiella pneumoniae*	Clinical isolates	Nutrient agar	37ºC

Table 2 Antimicrobial effect of *Blumea lacera* extracts (Zone of inhibition in mm)

Extract	*S. aureus*	*E. coli*	*S. typhi*	*S. pyogenes*	*K. pneumoniae*
Cold Water	++	++	++	++	+
Hot water	+++	+++	++	+++	+++
Cold Methanol	–	–	–	–	–
Hot Methanol	_	+	_	+	+

Key:
- No zone of inhibition
+ Zone of inhibition upto 8mm
++Zone of inhibition 8mm-12mm
+++ Zone of inhibition more than 12mm

Preliminary phytochemical analysis

Phytochemical screening of plant extract of *Blumea lacera* showed the presence of various constituents. The preliminary phytochemical tests result indicates the presence of tannins, saponins, flavonoids, phenols, and alkaloids. The qualitative test of phytochemicals was initially done by biochemical tests.

Table 3 Presence of phytochemicals using preliminary test

Phytochemicals	Results
Saponins	-
Tannins	+
Glycosides	-
Phenols	+
Flavanoids	+
Alkaloids	+

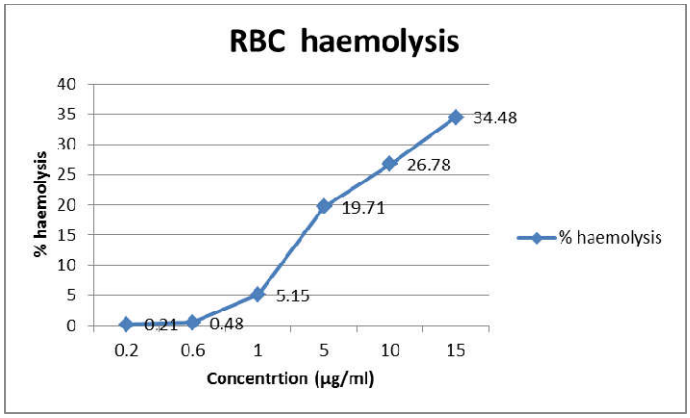
Keys:
+ Presence of the compounds
- Absence of the compounds

RBC haemolysis

O.D taken at – 560nm
Positive control- 2.9365
Negative control- 0.0437

Table 4 Calculation of Haemolysis % for various *Blumea lacera* concentrations.50 % haemolysis was not obtained at till 15mg/ml.

Concentrations (mg/ml)	Colour Blank	Test	% Haemolysis
0.2	0.0413	0.0913	0.21
0.6	0.0817	0.1395	0.48
1	0.2136	0.4063	5.15
5	0.8168	1.4306	19.71
10	1.4126	2.2312	26.78
15	2.2101	3.2514	34.48



Graph 1 Graph of RBC Haemolysis activity of *Blumea lacera*

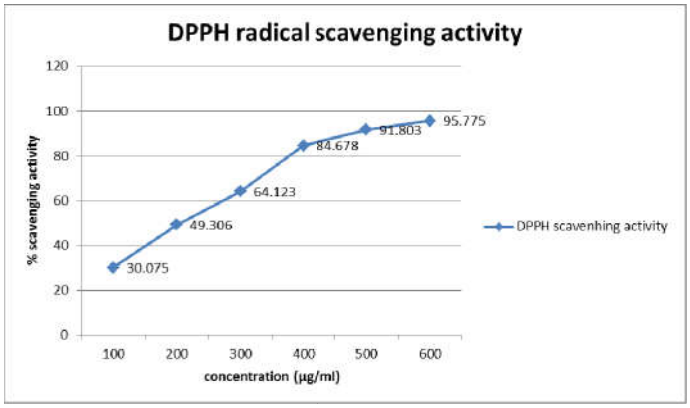
The *Blumea lacera* shows haemolysis activity i.e. 34.48% at 15mg/ml which is less than 50%. Thus it cannot be irritant or very irritant. It can be slightly, moderately or non irritant.

DDPH assay:

O.D of control – 1.586
Wavelength – 517 nm

Table 5 Calculation of antioxidant (DPPH radical scavenging) activity

Concentration(µg/ml)	Test	Radical scavenging (%)
100	1.109	30.075
200	0.804	49.306
300	0.569	64.123
400	0.243	84.678
500	0.130	91.803
600	0.067	95.775



Graph 2 Graph of antioxidant (DPPH radical scavenging) activity of *Blumea lacera*

IC50 Value

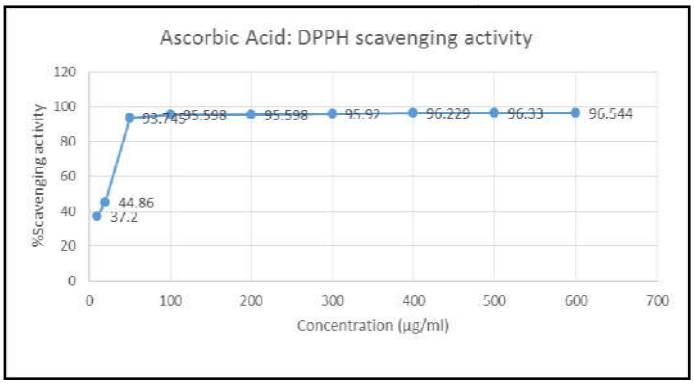
50 = 0.13616 x + 21.639
Therefore, x = 208.383
Thus, 50% antioxidant (DPPH radical scavenging) activity of *Blumea lacera* is seen at 208.383µg/ml concentration.

Standard Ascorbic ACID

O.D of CONTROL – 2.5461
WAVELENGTH – 517 nm

Table 6 Calculation of antioxidant (DPPH radical scavenging) activity of Ascorbic Acid

Concentration (µg/ml)	Test	Radical Scavenging (%)
10	0.9996	37.200
20	0.8744	44.86
50	0.0992	93.745
100	0.0698	95.598
200	0.0672	95.598
300	0.0647	95.920
400	0.0598	96.229
500	0.0582	96.330
600	0.0548	96.544



Graph 3 Graph of antioxidant (DPPH radical scavenging) activity of ascorbic acid

New strategies for antibiotic discovery or new alternatives to control bacterial infection by evading fast evolving resistance are highly desirable. Plants are known to have therapeutic values to treat various infections and diseases for centuries. Plants can produce complex mixtures of different compounds, some of which are being reported to have high antimicrobial activity against several important clinical pathogens (Vilas *et. al*., 2013).

The antibacterial activity of the tested ethanol and methanol extracts of different parts of *B. lacera* was more effective against the growth of Gram-positive compared to the Gram-negative bacteria. Gram negative bacteria have a lipopolysaccharide outer membrane through which entry of molecules is achieved based on their size and shapes. Most of the compounds present in aqueous and methanolic extracts probably could not pass through the outer membrane to reach their site of action resulting in less inhibition (Sakee *et.al*., 2011; Silvia, *et. al*.2013; Jahan *et. al*., 2014). There was significant antibacterial activity in ethanol extract of all the plant parts tested (Table 2). The zone of inhibition measured with ethanolic extract of root and leaf was highest against *S. aureus* which was more than any of the known antibiotics used in this study. Though, *B.lacera* ethanol and methanol extracts were showing significant inhibition of gram negative bacteria as compared to few other antibiotics used in the present study.

In this study, haemolytic activity of the aqueous extract of leaves of *Blumea lacera* was screened against normal human erythrocytes. Haemolytic activity of the plant is expressed in percentage. Samples exhibited very low haemolytic effect toward human erythrocytes. However, these extracts showed dose dependant increase in haemolytic activity (Table.4 Graph.1). Some other plants also have been studied for the haemolytic activity towards humans or animal erythrocytes. Different solvent extracts of *Syzigum cuminii* seeds and *Crateva nurvula* bark were reported to possess no haemolytic effect on sheep erythrocytes (Mathur *et.al*., 2011). Mukherjee and Rajasekaran (2010) reported the high haemolytic activity of the different solvent extracts of *Allium stracheyi* Baker towards human red blood cells. Aqueous extract of the leaves of *Aerva lanata* Linn., *Calotropis gigantea* Linn.and *Elaeocarpus ganitrus* Roxb were screened for the haemolytic activity towards human Erythrocytes (Bhaskara Rao *et.al*., 2011).

Scavenging activity for free radicals of 1.1-diphenyl-2-picrylhydrazyl (DPPH) has been widely used to evaluate the antioxidant activity of natural products from plant and microbial sources. The DPPH radical scavenging activity obtained in a study on *Blumea lacera* showed that the methanolic extract had approximately a DPPH radical scavenging activity with IC50= 208.383 µg/ml(Table.5&6 Graph.2&3). Several investigations are performed on different plant extracts for identifying their antioxidant activity. The methanolic extract of *Blumea balsamifera* showed an antioxidant activity with IC50= 398.16µg/ml (Bui *et. al*., 2017). So the *Blumea lacera* showed the significant antioxidant activity with IC50=208.383µg/ml.

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