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BLUMEA LACERA: ANTIOXIDANT, CYTOTOXIC AND PHYTOCHEMICAL ANALYSIS OF SOLVENT EXTRACTS

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ABSTRACT

In present study, the whole plant of *Blumea lacera* was investigated for their phytochemical, cytotoxic and antioxidant properties. Solvent extracts of the plant revealed the presence of terpenoids, flavonoids, tannin and sapponins in a number of extracts. Cytotoxic property of the sample was done using Brine shrimp lethality bioassay where hexane and chloroform showed significant activity (100%) at a highest dose of 200microgram/ml. Antioxidant activity was conducted qualitatively and quantitatively both. In qualitative TLC based antioxidant assay, maximum number of antioxidant bands was obtained in chloroform extract of EMW solvent. For quantitative antioxidant analysis FRAP (Ferric reducing antioxidant power assay) and DPPH(1,1-diphenil-2-picrylhydrazyl)radical scavenging assays were explored. Methanol extracts exhibited significant activity in both the assays.

KEYWORDS: *Blumea lacera*, medicinal plant, phytochemicals, cytotoxic, antioxidant, TLC, DPPH radical scavenging assay, FRAP.

INTRODUCTION

Blumea lacera belongs to *Asteraceae* family which consists of a large number of medicinally important plants.[1] Genus blumea consists of more than 100 species and a majority of them are used in many popular systems of medicine including Ayurvedic, Homoeopathy, Yunani, Chinese and Indonesian system of medicines.[2,3] It is a genus of mostly aromatic, woolly or pubescent herbs, under-shrubs or shrubs.

Blumea lacera is traditionally employed as an astringent in hemorrhages, and as de obstruent and stimulant. It is also applied for mumps, pneumonia. hepatitis. skin itch, bronchitis and inflammation of oral cavity.[4] In the Philippines a decoction of the fresh flowers is given before meals for bronchitis. Expressed juice of the leaves is useful anthelmintic, especially in cases of threadworm, either internally or applied locally. The expressed juice of the leaves, mixed with black pepper, is given for bleeding piles.[5] An astringent eye-lotion has also been prepared from the leaves and the plant is used as a diuretic, as it increases the flow of urine; it is prescribed as an antiscorbutic in West Africa.[6] In eastern Nepal, *Blumea lacera* is known as Gangansu and its Root paste is applied on and round swelling region to prevent from cutaneous infection. In the Konkan region of India, the plant is used to drive away fleas and other insects. In Chandigarh region, it is used in bleeding piles. It is used externally in treatment of cancerous wounds. *Blumea lacera* is used in folk medicine for the treatment of cough, bronchitis, dysentery and wound healing.[7] In the present study locally available plants were explored for their cytotoxic, antioxidant and phytochemical properties so as to ascertain the medicinal value of the same.

MATERIALS AND METHODS

Collection and processing of plant materials

Whole plant samples of *Blumea lacera* were collected from the campus of Regional Plant Resource Center (RPRC), Bhubaneswar. Samples were thoroughly washed under tap to remove dust and soil particles, dried in shade and were made into fine powder using mechanical grinder of Lexus make. Moisture content of the plant was calculated by using the following formula:

$$\textbf{Moisture content (\%)} = \frac{Fw - Dw}{Fw} \times 100$$

Where, Fw = Fresh weight of plant sample

Dw = Dry weight of powdered plant sample

Solvent extraction was done by soxhlet extraction method. Percentage yield of all the four extracts was also calculated using the following formula.

$$\textbf{\% of yield} = \frac{Extract\ weight}{Powdered\ Weight} \times 100$$

Phytochemical analysis of solvent extracts

Phytochemical analysis was conducted using standard protocols.[8] Brief methodology for phytochemical analysis of the different metabolites was as follows:

1. Test for Alkaloids: Alkaloid tests were done by using 3 different reagents.

- **Dragendroff's test -** To 1ml of extract 2ml of 1% HCl was added and then boiled for few minutes, after boiling 2-3 drops of dragendroff's reagent was added & sample was observed for reddish brown precipitate.
- **Wagner's test -** To 1ml of extract 1ml of 1% H_2SO_4 was added followed by few drops of wagner'sreagent. Formation of precipitate depicts the presence of alkaloids.
- **Mayer's test -** To 1ml of extract, 2ml of 1% HCl and mayer's reagent was added dropwise and was observed for the formation of precipitate.

2. Test for Flavonoid: To 2.5 ml of extract, 1 ml of 10% NaOH was added. From the side of the test tube, drops of conc. HCl were added. Yellow colour turns to colourless which indicates presence of flavonoids.

3. Test for Anthraquinone: To 1ml of extract 2ml of 5% of KOH was added and was observed for pink colouration.

4. Test for Saponin: To 1ml of extract 2ml of $NaHCO_3$ was added and on shaking forms lather.

5. Test for Terpenoids: To 1ml of extract 400µl of chloroform and 4-5 drops of conc.H_2SO_4 was added from the walls of the test tube. A reddish brown ring indicates the presence of terpenoids.

6. Test for Cardiac glycoside: To 2.5ml of extract 2ml of glacial acetic acid**,** few drops of $FeCl_3$ and conc.H_2SO_4 was added from the walls of the test tube. Presence of cardiac glycoside is determined by reddish brown ring.

7. Test for Tannin: It can be observed by two methods.

- **Method A -** 1ml of extract was boiled and few drops of $FeCl_3$ were added to it. The sample was observed for blue, black, green colour.
- **Method B -** To 1ml of extract 500µl of lead acetate was added which gives yellow colour.

8. Test for Starch: To 1ml of extract 500µl of iodine was added, which results in blue coloration.

9. Test for Phlobatannin: To 1ml of extract 1% HCl was added and boiled, formation of precipitate occurs on positive test.

Cytotoxic activity

Brine shrimp *(Artemia salina*) mortality assay

Cytotoxic activity study was carried out by brine shrimp lethality assay using standard protocols.[9] Here simple zoological organism (*Artemia salina*) was used as a convenient monitor for the screening. Brine shrimp (*Artemia salina*) eggs were hatched in artificial sea water, which was prepared using black salt 3.6 gm/ 200 ml distilled water. The eggs were incubated for 48 hours at temperature of about 28° C to get the desired growth of the larvae for biological evaluation. For each dose level 3 replicates were used. To each test tube of negative control, positive control and extracts, 20 numbers of brine shrimp larvae were taken and volume was made up to 10ml by adding salt water. Cytotoxic assay was carried out at four doses 25, 50, 100 and 200µg/ml. Motility assessment of larvae was conducted at each hour up to four hours.

Motility readings were graded as below.

4+ = high motile

3+ = motile

2+ = sluggish

1+ = slow

Nil = no activity

After 24 hrs, the number of survived larvae in the control and experimental tubes were counted. From this data, the percentage (%) of inhibition of the brine shrimp was calculated for each concentration using the following formula:

$$\textbf{Percentage Inhibition} = \frac{\textbf{No of larvae in control} - \textbf{No of larvae in experimental}}{\textbf{No of larvae in control}} \times 100$$

Antioxidant activity

Qualitative Analysis (TLC based antioxidant assay)

TLC is one of the most widely used and potent technique to resolve mixture of plant compounds. It is also called DPPH (2,2-Diphenyl 1-picrylhydrazyl) assay. TLC sheet (Silica gel 60 F_{254}, Merck company, Germany) was used as stationary phase. The developed TLC

plate was sprayed with 0.2% DPPH in methanol as an indicator as per the standard protocol.[10]

The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol.

$$DPPH + AH \longrightarrow DPPH{-}H + A^{-}$$

(Violet color) **(Yellow color)**

Three types of solvents were prepared for TLC chromatography technique.

1. BEA – Benzene: Ethanol: Ammonium hydroxide(90:10:1) [Non polar/Basic]

2. CEF – Chloroform: Ethyl acetate: Formic acid(5:4:1) [Intermediate polarity/Acidic]

3. EMW – Ethyl acetate: Methanol: Water(40:4.5:4) [Polar/Neutral]

Qualitative screening of the constituents in each of the plant extract of *Blumea lacera* for antioxidant activity was done by TLC analysis. The precoated TLC sheets were activated at 100^0C for 2 minutes. The samples were spotted with the help of micro tips leaving 1cm from the bottom of the sheet. After drying of sheets DPPH solution was sprayed. Yellow bands on purple background represent the antioxidant bands of the extract.

Rf values of all the antioxidant bands were calculated using the following formula.

$$\textbf{Retardation factor } (R_f) = \frac{\textbf{Distance travelled by the compound}}{\textbf{Total Distance travelled by the solvent}}$$

Quantitative anti-oxidant Analysis

Quantitative analysis was done by two popular methods as follows;

DPPH free radical scavenging assay

For DPPH free radical scavenging assay 1mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution was prepared by adding 4mg of DPPH dissolved in 10ml methanol. DPPH assay was done by serial dilution method starting from concentration of plant extracts (7.8 µg/ml, 15.62 µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml, 250 µg/ml, 500µg/ml, 1000µg/ml) was prepared in methanol. 1ml of each sample was taken in the test tubes and 500µl of DPPH solution was added. For control, each test tube contained 1ml methanol and 500µl DPPH**.** Samples were incubated for 30 minutes at room temperature in dark. All the samples were taken in triplicate and complete set of experiment was repeated three times. Optical density (OD) was measured at 517nm in spectrophotometer.[11] The percentage of free radical scavenging activity was calculated from the following formula:

Percentage free radical scavenging [DPPH] = [(Ac – As) ÷ Ac] × 100

Where, Ac = Absorbance of control and As = Absorbance of sample.

FRAP ASSAY (Estimation of total antioxidant activity)

Total antioxidant activity is measured by ferric reducing antioxidant power (FRAP) assay as per the standard protocols.[12] Spectroscopic method is based upon the ability of antioxidants to reduce Fe^{+3} to Fe^{+2} in the presence of TPTZ, forming an intense blue Fe^{+2}-TPTZ complex with an absorption maximum at 593 nm. The decrease in absorbance is proportional to the antioxidant present. The FRAP reagent (300 mM Acetate buffer pH 3.6: 40 mM Dilute HCl: 10 mM TPTZ: 20 mM $FeCl_3.6H_2O$ in the ratio of 10: 1: 1) was prepared and then incubated at 37^oC in a water bath for 10 minutes. Absorbance of FRAP reagent was taken at 0^{th} minute (t_0) which was the control of the experiment. Ascorbic acid was taken as standard. A total of 100 µL of sample/standard and 300µl of distilled H_2O was then added to the FRAP reagent and incubated at 37°C for 4mins (t4). A reagent blank was prepared as described above but 100µl of distilled H2O was added instead of test sample. Duplicate test tubes were taken and absorbance was measured at 593nm. Ascorbic acid was taken as standard and 1.0mM to 0.1mM concentration of standard was prepared for the FRAP assay and based on the observations a standard curve was plotted. A number of dilutions of each sample extract were tested allowing dose response curves to be produced. The FRAP values were expressed in mmol Ascorbic acid equivalents (AAE).

RESULTS AND DISCUSSIONS

Moisture content of *Blumea lacera* whole plant was 72.4 percent and as can be seen in Table 1, methanol extract showed maximum yield suggesting that polar molecules outnumber the non polar.

Table 1: Percentage of yield.

Extract	Weight of sample	% of yield
Hexane	1.08gm	4.5%
Chloroform	0.504gm	2.1%
Acetone	0.321gm	1.3%
Methanol	2.312gm	9.63%

Phytochemical analysis of whole plant of Blumea lacera

Amongst all the extracts, methanol extract was most abundant and showed the presence of flavonoids, sapponins, terpenoids, Cardiac glycosides and tannins. Presence of flavonoids, a

very important medicinal candidate with antioxidant activity is in confirmation with earlier studies.[13] As can be seen in Table 2 Terpenoids was present in three out of four extracts. Terpenoids are responsible for a large number of biological activities like anti inflammatory and antimicrobial.[14] Thus presence of same indicates the biological potential of the plants.

Table 2: Phytochemical analysis of extracts of *Blumea lacera*.

Metabolite	Hexane extract	Chloroform extract	Acetone extract	Methanol extract
Alkaloids	-	-	-	-
Flavonoid	-	-	+	+
Anthraquinones	-	-	-	-
Saponin	+	+	-	+
Terpenoid	+	-	+	+
Cardiac glycoside	-	-	-	+
Tannin	-	+	-	+
Starch	-	-	-	-
Phlobotanin	-	-	-	-

Anti-oxidant Activity of *Blumea Lacera*

Qualitative Antioxidant Screening

In this test all the extracts were run in three different solvents i.e. CEF, BEA and EMW. DPPH is reduced and resulting molecule gives a yellow band when chromatograph is sprayed with 0.2% DPPH solution. Best separation was obtained in EMW solvent where all the extracts showed maximum number of antioxidant bands with maximum number of 9 bands in the chloroform extract. Number of bands is directly corelated with the extent of antioxidant activity of the extract.

Table 3: Qualitative TLC analysis of solvent extracts.

Samples	Solvent	No. of bands	R_f VALUES
Ascorbic acid	BEA	1	0.22
	CEF	1	0.76
	EMW	1	0.10
Hexane	BEA	6	0.07,0.12,0.19,0.36,0.42,0.53
	CEF	3	0.06,0.17,0.25
	EMW	7	0.06,0.20,0.35,0.42,0.48,0.67,0.87
Chloroform	BEA	5	0.10,0.18,0.33,0.40,0.51
	CEF	6	0.14,0.23,0.35,0.43,0.5,0.54
	EMW	9	0.07,0.17,0.21,0.34,0.40,0.46,0.59,0.67,0.87
Acetone	BEA	5	0.09,0.27,0.33,0.48,0.84
	CEF	5	0.06,0.21,0.31,0.39,0.51
	EMW	3	0.09,0.18,0.23

Methanol	BEA	5	0.07,0.18,0.25,0.39,0.71
	CEF	3	0.18,0.31,0.53
	EMW	0	_

Quantitative Antioxidant Activity

DPPH Free Radical Scavenging Assay

The reactivity of different extract of *Blumea lacera* was analysed with DPPH, a stable free radical. As DPPH picks up one electron in the presence of free radical scavenger, the absorption decreased and the resulting discoloration was related to the number of electrons gained. The DPPH free radical scavenging activity of different extract (Hexane, Chloroform, Acetone, Methanol) was determined by the multiple reader in 517nm. *Blumea lacera* hexane extract exerted an inhibition of 64.23, chloroform extract exerted an inhibition 71.21, acetone extract exerted an inhibition of 78.52, methanol extract exerted an inhibition of 89.65 and that of ascorbic acid was 97.12 at 1000µl/ml.

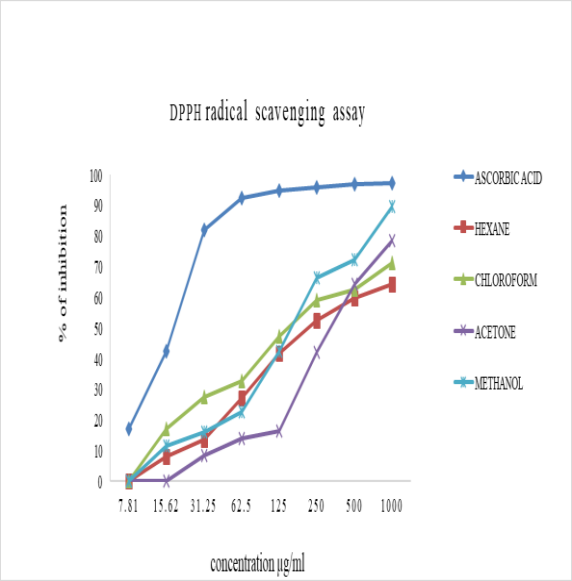


Fig 1: DPPH radical scavenging assay.

Ferric reducing antioxidant power assay

The antioxidant can donate an electron to free radicals, which leads to neutralization of the radical. Reducing power was measured by direct electron donation in the reduction of Fe^{3+} to Fe^{2+} in the presence of TPTZ. The product was visualized by forming an intense blue colour complex and then measured at 593nm. Highest Frap value was obtained in methanol extract (Fig 2)

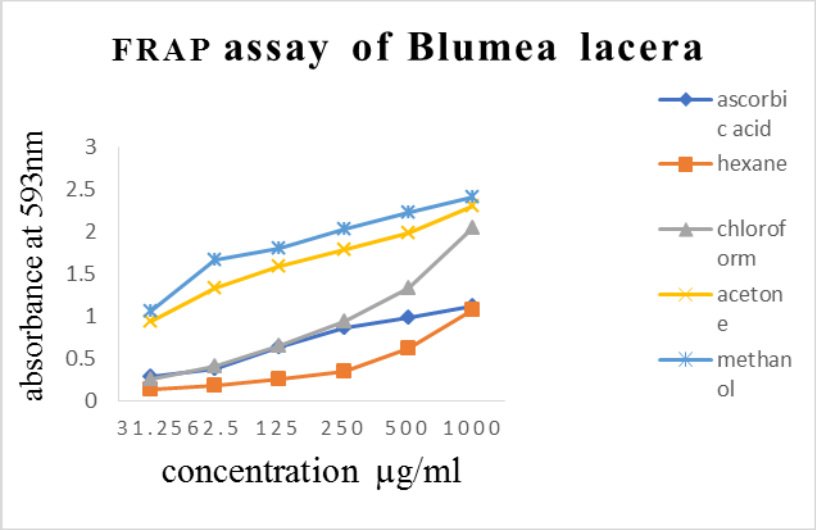
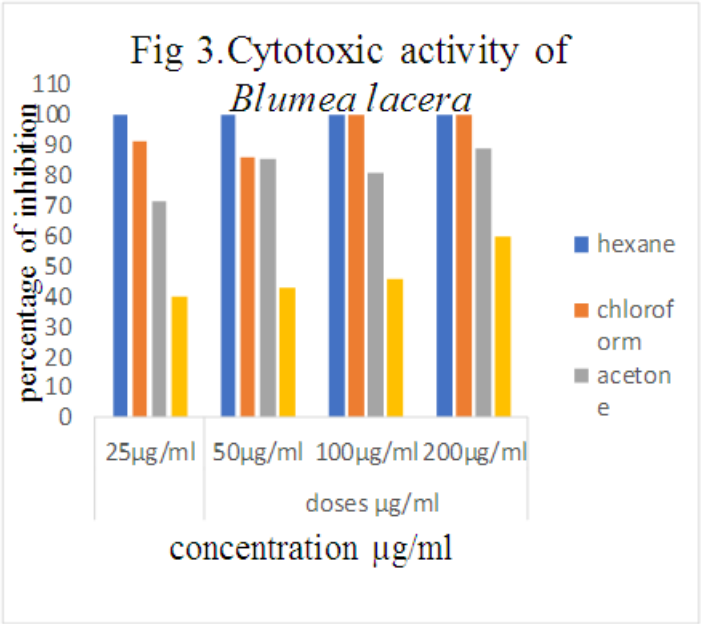


Fig 2: FRAP assay of solvent extracts of *Blumea lacera.*

Cytotoxic activity of leaf extracts of Blumea lacera

All the extracts showed highly significant cytotoxic activity at higher dose of 200microgram /ml. As can be seen in the Fig 1, hexane extracts showed maximum activity followed by chloroform extract. Except in chloroform extract activity was dose dependent. As brine shrimp assay has a good correlation with anti- inflammatory and anticancer activity[9] hence hexane and chloroform extract have provided a lead for the development of anticancer agent.



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