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BARLERIA CRISTATA: A COMPARATIVE ANALYSIS OF PHYTOCHEMICAL, CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF LEAF AND BARK EXTRACTS

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ABSTRACT

Phytochemical, cytotoxic and antioxidant profiles of leaf and bark of the ornamental plant *Barleria cristata* was explored in the present study. Hexane, Chloroform, Acetone and Methanol solvents were prepared using successive extraction method. All the extracts were tested for their cytotoxic activity using Brine shrimp lethality assay. Antioxidant activity of the extract was assessed using qualitative and quantitative assays. For qualitative, Thin layer chromatography based DPPH assay and for quantitative, Nitric oxide radical scavenging, DPPH radical scavenging and Ferric reducing antioxidant power (FRAP) were conducted. Leaf and bark extracts were found to contain terpenoids, anthraquinones, alkaloids, flavonoids and saponins. Methanol extract of *Barleria cristata* leaf showed potent bioactivity/cytotoxic and antioxidant activity.

KEYWORDS: Cytotoxic, brine shrimp, antioxidant, DPPH.

INTRODUCTION

Barleria cristata is an ornamental perennial shrub belonging to Acanthaceae family. Same has been reported to possess antimicrobial, anti- inflammatory and hepatoprotective activity,[1, 2] Another shrub from the same family *Barleria prionitis*, has been much widely researched with documented medicinal properties of the whole plant, leaves, and roots against e.g., diabetes and respiratory diseases.[3] Because of its antiseptic properties, extracts of the plant are incorporated into herbal cosmetics and hair products to promote skin and scalp health.[4] Its leaves are known to contain the flavonoid 6-hydroxyflavone (6HF) - a

noncompetitive inhibitor of the protein Cytochrome P450-2C9. Iridoid glycosides have also been reportedly isolated from aerial parts of *Barleria prionitis*,[5] Keeping in view of the excellent properties of species of same genus, in the present study aerial part of *Barleria cristata* were exoplored for their cytotoxic, antioxidant and phytochemical properties followed by bioassay guided fractionation to get active ingredients of the plant.

MATERIALS AND METHODS

Plant materials were collected from Medicinal Germplasm garden of Regional Plant Resource Centre, Bhubaneswar, Khurda district, India. Leaves were washed, shade-dried, ground into fine powder and 30g of coarsely powdered leaves were successively extracted using Soxhlet apparatus.[6] while the bark was subjected to successive cold maceration in 1:3 volume. The solvents used were Hexane, Chloroform, Acetone and Methanol in the order of lower polarity to higher. The extracts were concentrated using a Buchii R-200 Rotavapor apparatus. The concentrates were left to dry further and then solvent-free extracts were stored in screw-cap vials for future use.

Phytochemical Tests

Tests for presence of various classes of phytochemicals, namely alkaloids, flavonoids, saponins, tannins, phlobatannins, terpenoids, cardiac glycosides and anthraquinones were carried out using standard protocols.[7]

Brine Shrimp Lethality Assay

Assay was conducted as per the standard protocols.[8] Brine shrimp cysts were incubated at 28±2^{o}C for 48h under constant aeration. On day 3, larvae (nauplii), were counted to 20 numbers and added into vials marked as negative control, positive control and sample respectively and after adjusting total volume to 10mL with brine then co-incubated with doses of (25, 50, 100 and 200 µg/mL of sample (extracts dissolved in ethanol), for 24h.

Motility of the larvae was observed at 1 hour interval up to 4 hours. After 24 hours, final count of live nauplii in each vial was taken, and percentage of inhibition calculated by comparing treated samples with the controls. Lethality was calculated as

$$\%\ inhibition = \frac{live\ nauplii\ in\ control\ -\ live\ nauplii\ in\ sample}{live\ nauplii\ in\ control} * 100$$

Thin-layer Chromatography-based Qualitative antioxidant assay

Extracts were diluted and run on pre-coated TLC Silica gel 60 F_{254} sheets (Merck, Darmstadt) in solvent systems ranging from basic to neutral to acidic pH, and intermediately polar to polar nature were used namely, BEA (Benzene:Ethanol:Ammonium hydroxide) in the ratio 45:5:0.5 (Basic/intermediately polar), EMW (Ethyl acetate:Methanol:Water) in the ratio 40:5.4:4 (Neutral/polar) and CEF (Chloroform:Ethyl acetate:Formic acid) in the ratio 5:4:1 (Acidic/polar). DPPH (HiMedia, Mumbai) was used as spraying agent,[8] and L-ascorbic acid was used as standard.

The TLC sheet was marked for antioxidant (yellow) bands and Retardation factor (R_f) value was calculated for each band as, $R_f = \frac{distance\ travelled\ by\ solute}{distance\ travelled\ by\ solvent}$

Quantitative antioxidant assay

DPPH radical scavenging

1mM DPPH solution in Methanol was added to various concentrations of plant extracts (62.5, 125, 250, 500 and 1000 µg/mL) followed by incubation in dark for 30 min at room temperature, the intensity of yellow color chromophore formed was measured at 517 nm.

% DPPH scavenging = $[(A_{control} - A_{sample})/A_{control}] \times 100$

Nitric-oxide Reducing Assay

Different concentrations of sample extracts (25, 50, 100, 200, 400 and 800 µg/mL) were incubated with Sodium nitroprusside (Nitric oxide radical generator) in phosphate buffered saline. After 150 minutes of incubation Griess reagent was added to develop color and absorbance was measured at 546 nm. Quercetin was used as standard.

Ferric Reducing Antioxidant Power (FRAP) Assay

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 0th 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 370C 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, expressed in mmol Ascorbic acid equivalents (AAE)/gram sample in dry weight were derived from a standard curve.[9]

RESULTS AND DISCUSSIONS

Solvent extracts of *Barleria cristata* showed variable yield on extraction. The yield of Chloroform extract was highest at 7.86% followed by Hexane extract at 6.42% for leaf, while for bark, the yield of Chloroform extract was 5.71% followed by Hexane extract at 1.52%. Although yield of chloroform and hexane was more yet acetone and methanol extracts were rich in important class of compounds like flavonoids, saponins and terpenoids (Table1). This study is in confirmation with earlier study.[10] Presence of the above class of compounds is indicative of the medicinal potential of the plant as all the major class of compounds as above are known for their anticancer, anti inflammatory and antirheumatic properties.[11,12]

Table 1 : Phytochemical profile of solvent extracts of *Barleria cristata*

Phytochemical	Hexane		Chloroform		Acetone		Methanol	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
Alkaloids	-	-	+	-	-	-	-	-
Flavonoid	-	-	-	-	+	+	+	+
Saponin	-	-	-	-	+	+	+	+
Tannin	-	-	-	-	-	-	-	-
Terpenoid	-	+	+	-	+	+	+	+
Cardiac glycoside	-	+	-	+	-	+	-	+
Phlobatannins	-	-	-	-	-	-	-	-

Cytotoxic activity

Brine shrimp (*Artemia salina*) lethality assay is a widely used method to screen for bioactive compounds from medicinal plants that are candidates for anticancer treatment. It requires low resources, is quick and produces statistically significant results.[13,14]

Majority of the extracts showed dose dependent activity, Methanol extract of both leaf and bark showed highest activity to the tune of 94% and 83% respectively(Fig 1 &2).

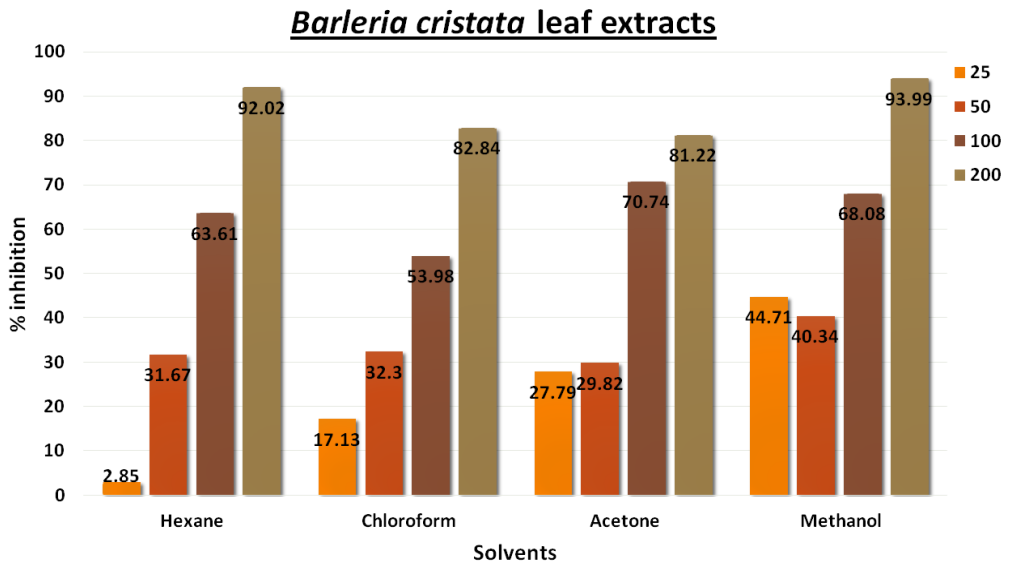


Figure 1 Brine shrimp lethality assay of leaf extracts.

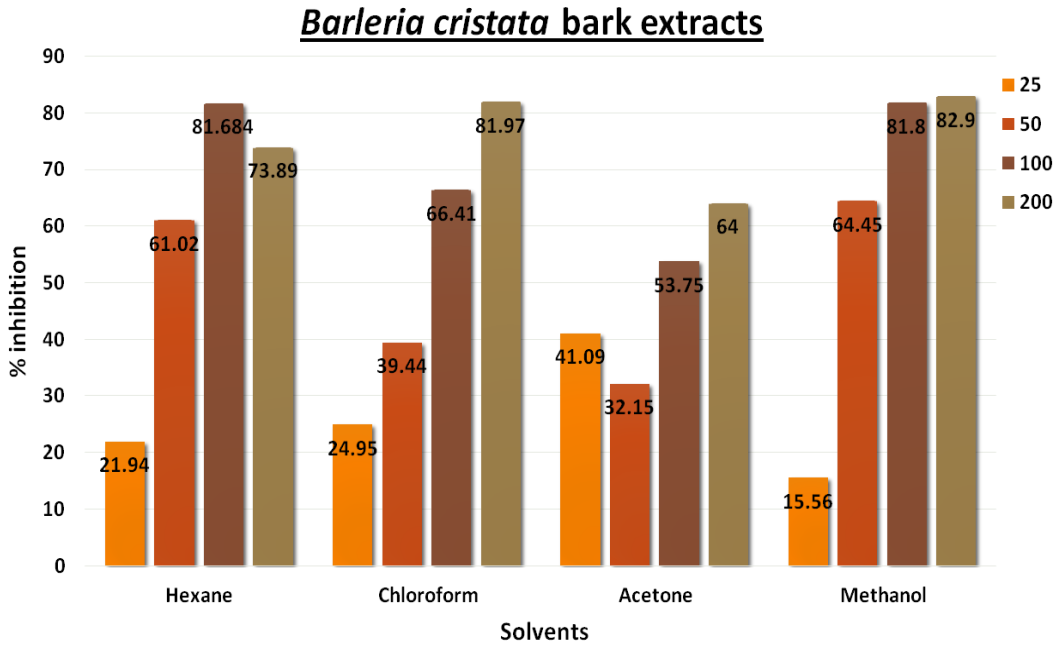


Figure 2 Brine shrimp lethality assay of bark extract

Antioxidant assays

Qualitative as well as quantitative assays both are based on radical scavenging activity of the molecules present in the extracts.

DPPH Assays

2,2-diphenyl-1-picrylhydrazyl (DPPH) ($C_{18}H_{12}N_5O_6$: Mr = 394.33) is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. The color

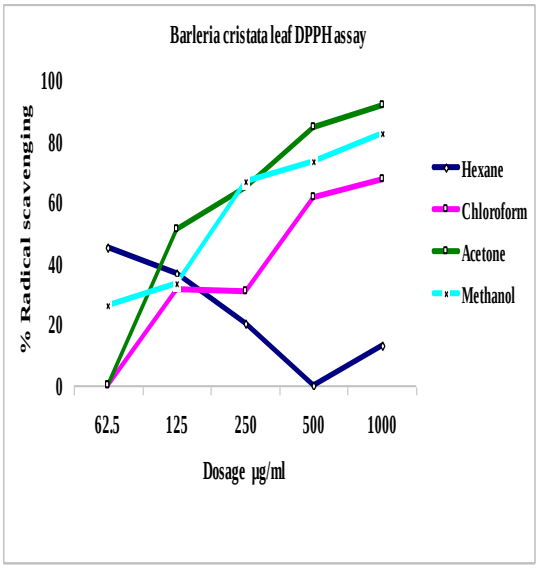
of DPPH solution in methanol, upon encountering an antioxidant, changes from deep purple to yellow, the intensity of which depends on the amount and nature of radical scavenger present.

Qualitative TLC based and Quantitative DPPH Assay

As in Table 2, all the extracts showed large number of antioxidant bands suggesting a number of antioxidant molecules in the extract. Leaf extracts showed more anti oxidant bands in comparison with the bark extracts same result was validated with the quantitative DPPH radical scavenging assay in which acetone leaf extract showed more than 80% radical scavenging (Fig 3) where as none of the bark extracts showed more than 60% radical scavenging (Fig 4).

Table 2. Qualitative TLC-based DPPH antioxidant assay:

Extract/System	BEA		EMW		CEF	
	Leaf	Bark	Leaf	Bark	Leaf	Bark
Hexane	6	2	1	1	1 + Streak	1
Chloroform	9	5	5	6	7	6
Acetone	5	5	5 + Streak	4	6	3
Methanol	5	0	5	1	8	1



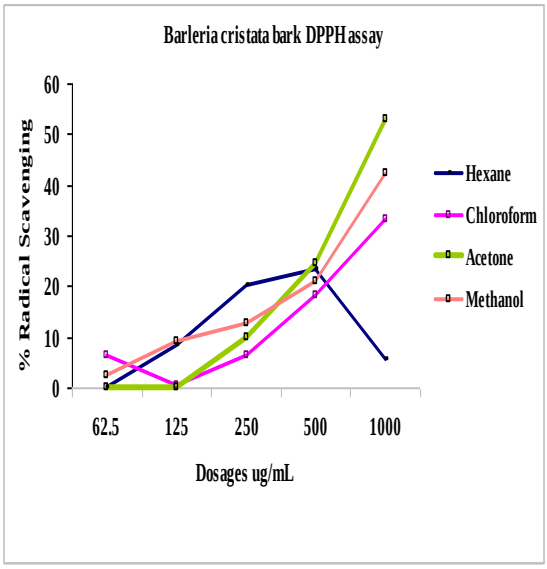


Fig 3 & 4: DPPH radical scavenging assay of leaf and bark extracts of *Barleria cristata*.

Nitric oxide radical scavenging assay

Methanol extract of bark as well as leaf showed highest nitric oxide radical scavenging activity amongst all other extracts, but methanol extract of bark was more potent(>80%) in

comparision to that of leaf which showed mild activity(<50%). Overall polar extracts had better antioxidant activity in comparison to non polar extracts.

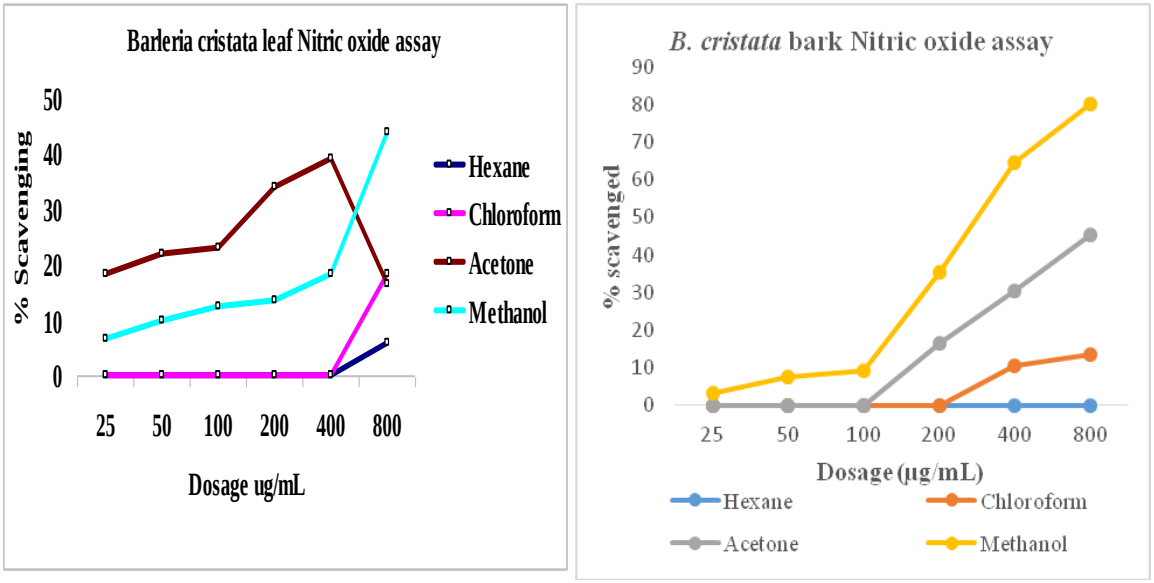


Fig 4 &5 Nitric oxide assay of leaf and bark extracts of *Barleria cristata*

CONCLUSION

Solvent extracts of *Barleria cristata* leaf and bark samples were rich in medicinally important class of compounds like saponins, flavonoids and terpenoids. Methanol and acetone extracts of both leaf and bark showed significant cytotoxic and antioxidant activities.

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