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ETHNOPHARMACOLOGICAL VALIDATION OF TRADITIONAL CLAIMS OF BARLERIA PRIONITIS, LINN FOR ITS ANTI-ASTHMATIC POTENTIAL

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Abstract

Barleria prionitis, Linn (BP) is well known ethno medicinal plant have been known for various traditional claims as well rich source of bioactive phytochemicals. It has been traditionallyused for the treatment of variety of respiratory disorders such asthma. However, it has not been scientifically evaluated for these pathological conditions evidences, this piece of research is an attempt validate and corroborate the same.

BP whole plant was successively extracted with hexane, chloroform, acetone and ethanol. Obtained extracts were screened for phytochemical profile, these extracts were standardized for the content of TPC and TFS. Furthermore these extracts were assessed for antioxidant potential using DPPH and ABTS assay. % histamine release was calculated as an indication of mast cell stabilization. Finally extracts were screened for broncho-relaxation effect against histamine induced tracheal contraction which was expressed as % broncho-relaxation.

Successively obtained extracts of whole plant of BP showed presence of prominent phytochemical such as alkaloids, polyphenols, steroids and terpenoids. Standardization of extracts revealed highest TFC and TPC in ethanol extract exhibiting 19.12 RE mg/g & 24.11 GAE mg/g as comparedto other extracts. When successively obtained extracts subjected to DPPH and ABTS assay BP-ET extract exhibited best radical scavenging effects with IC_{50} values of 36.39 &25.72 respectively.

Furthermore % histamine release in terms of mast cell stabilization exhibited by ethanol extract was 10.96 at 50 mg/ml. Finally, among all extracts screened for broncho relaxant effect against histamine induced contraction, BP-ET showed 94.12% relaxation at 0.6ml.

Overall finding of present work concludes that, successively obtained whole plant BP extract revealed presence of prominent phytochemicals such as alkaloids, polyphenols, steroids and terpenoids. These phytochemicals attributes for its overall anti-oxidant, mast cell stabilization and broncho-relaxant effects.

Key Words: *Barleria prionitis,* Linn, Mast cells, Standardization, Broncho relaxation

INTRODUCTION –

The porcupine flower plant *Barleria prionitis*, Linn is a member of the genus Barleria and family Acanthaceae. Its native to tropical regions of Africa and Asia (specifically India), but can also be found in many other Asian countries such as Bangladesh, Malaysia, Pakistan, Philippines, Sri Lanka and Yemen (Shendage & Yadav 2010; Ambalwad VD 2021). It is an upright, perennial, thorny, evergreen shrub that typically grows from a single taproot to a height of 1.5 metres. Lateral roots that spread out in every direction. The leaves have an oval shape that is narrow at both ends, measuring up to 100 mm in length and 40 mm in width (ellipsoid). Three to five spiky, pale-colour, 10–20 mm long spines guard the base of the leaves. The tubular yellow-orange flowers have multiple long filaments that stick out. At the top of the plant, flowers are tightly clustered together, although they can also be found individually at the base of leave. The oval-shaped seed capsule contains two very big, flat seeds that are protected from the elements by matted hairs and a sharp beak. The branches and stems are smooth, rigid, and have a light brown to light grey colour (Nadkarni, 1954; Kirtikar & Basu 1935).

Many potential ethno medicinal uses are reported for prionitis species of the genus Barleria such as to cure catarrhal affections, the entire plant or certain portions (leaf, stem, root, bark, and flower) have been used, to treat ulcer, whooping cough, glandular swellings, inflammations, urinary tract infection, fever, jaundice, stomach ailments, along with as a tonic and diuretic. In addition, it is used to treat dropsy, hepatic obstruction, jaundice, urinary infections and boils (Nadkarni 1954, Khare CP 2008). The paste made from the roots is also used to treat glandular swellings and boils. Additionally, it has been reported to treat bacterial illnesses, toothaches, and anemia. In particular, the flower are well known for treating toothaches and bleeding gums. Owing to its anti-odontalgic characteristic, it is also widely recognized as "Vajradanti." Leaves are used by some tribal communities as an irritant and to treat piles. Some traditional claims also found its use to treat sciatica, scrotal enlargement and limb stiffness.

Asthma is a chronic inflammatory illness that affects the lungs' airways, which is typified by reversible airflow obstruction, triggered bronchospasms, with variable and recurrent symptoms. One of the major cause of increased incidence of respiratory illnesses and the financial burden has been reported to be associated with to India's continued rapid urbanization. It is believed that a mix of environmental and genetic factors cause asthma. Although asthma cannot be cured but it can be managed with proper monitoring of medications and minimization of exposure to triggering factors (Cote A 2020). Existing treatments for asthma are incomplete and may raise safety concern in chronic administration as it usually lead to resistance as well dependence for steroid as well as inhaler abuse which mainly pose limitations to existing clinical practice (Patel VH et al 2023).

India is treasure trove of ethno medicinal plants and establishment of practical knowledge with scientific evidences for these medicinal plants is prime duty of ours. In an attempt to do so, this research is undertaking to corroborate the traditional claim of BP to treat asthma and associated complications such as stress reduction, management of inflammation and subdue allergic reaction.

MATERIALS AND METHODS

Plant materials

The flowering plant of *Barleria prionitis* was collected from the outfield of Nanded city, Maharashtra, India. The plant material was authenticated by, Department of Botany, Shree Renuka Devi Mahavidayalaya, Mahur, Dist Nanded, MS, India (PhD. 2019-20/1015). The plant material was coarsely powdered so as to facilitate further extraction.

Chemicals and reagents:

All the chemicals purchased are of Loba Chemicals. For extraction, phytochemical analysis and TLC chemicals were purchased of synthetic grade, while for standardization and other *in vitro* as well as *in vivo* analysis chemicals purchased were of analytical grade.

Instruments:

UV-Visible spectrophotometer, Rotary evaporator, micro plate reader (iMark, BioRad).

Animal Husbandry:
Animals were acclimatized to the laboratory conditions for an appropriate period and provided with standard laboratory animal feed and drinking water *ad libitum*. Animals were grouped as per requisite of animal experimentation.

Experimental Methodology
Preparation of extract of *Barleria prionitis* L
The ground powdered material (500g) were exhaustively defatted with n-hexane by Soxhelet extraction followed by successive extraction with chloroform, acetone and finally with absolute ethanol. Obtained extracts were collected, filtered and concentrated over rotary evaporator. Resulting concentrated extracts were marked and labelled as BP-HE, BH-CH, BP-AC and BP-ET respectively, these were stored in tightly closed glass container in desiccator for further use.

Evaluation of Phytochemical profile of *Barleria prionitis* L. whole plant successive extracts
Preliminary phytochemical profile was assessed for the presence of various phytoconstituents present in successively obtained BP extracts using routine test reported previously (Gokhale MS & Kokate CK, 2008).

Standardization of BP extracts for Total flavonoid content (TFC) & Total phenolic content (TPC)
TFC & TPC of BP extracts which shown positive test for presence of flavonoids, tannins or polyphenols respectively. These extracts were further assessed for content of TFC by the aluminum chloride colorimetric method using rutin as standard and expressed as rutin equivalent per g dry weight. TPC by the Folin–Ciocalteu colorimetric method using gallic acid as standard and expressed as mg/g gallic acid equivalent (GAE) on dry weight basis by means of method established and reported previously (Ghante M H et al 2012).

Evaluation of Antioxidant potential
DPPH radical stabilization
5µl of different stock of the test compound was added to 0.1 ml of 0.1mM DPPH solution in a 96 well plate. The reaction was set in triplicate form and duplicates of blank was prepared containing 0.2 ml DMSO/Methanol and 5µl compound of different concentrations. The plate was incubated for 30 min in dark. At the end of the incubation, the decolonization was read 495 nm using a micro plate reader (iMark, BioRad). Reaction mixture containing 20µl of deionized water was served as Control. The scavenging activity was presented as '% inhibition' with respect to control. IC_{50} was calculated using Software Graph Pad Prism 6 (Adeleke A et al 2020).

ABTS Radical stabilization
ABTS radicles were prepared by mixing APS (2.45 mM) and ABTS (7mM) solution, which was diluted 100X to prepare ABTS free radical reagent. Add 10µl of different stock of the standard (Ascorbic Acid) and samples to the 200µl of ABTS free radical reagent in 96 well plate and incubated at RT for 10 min in dark. After incubation measure absorbance of the decolonization at 750nm using a micro plate reader (iMark, BioRad). Results were presented with respect to negative control. IC_{50} was calculated using Software Graph Pad Prism 6 [Cao G and Prior RL 1998; Kambayashi Y et al 2009).

Evaluation of *in vitro* mast cell stabilization activity
This was conducted using the rat peritoneal mast cell (RPMC) degranulation model. Wistar rats were administered 50 ml of normal saline in the peritoneal cavity under anesthesia. The peritoneum

was then gently massaged for a few minutes to stimulate the release of mast cells. Subsequently, the peritoneum was incised to collect the fluid, which was centrifuged at 2000 rpm for 15 minutes. The supernatant was discarded, and the cells were re-suspended in normal saline. A 1 ml sample of the obtained mast cell suspension was incubated with various concentrations (50 and 100 mg/ml) of plant extracts (1 ml) at room temperature. After 20 minutes, 1 ml of compound 48/80 (10 µg/ml) was added, and the mixture was allowed to incubate at room temperature for an additional 20 minutes to induce histamine release. Subsequently, 0.4 ml of 1N NaOH and 0.1 ml of o-phthaladehyde (1% w/v) were added, and the mixture was incubated for 5 minutes. This was followed by the addition of 0.3 ml of 3N HCl. The fluorescence of the resulting mixture was recorded using a spectrofluorometer at an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Sodium cromoglycate (50 & 100 µg/ml) served as the standard drug. The amount of histamine released was calculated from the corresponding fluorescence using the standard plot of histamine hydrochloride (Ghante M H et al 2012).

Broncho dilation evaluation of extracts *in vitro*

This study investigated the trachea-relaxant effects of various concentrations of the extract of BP and compared with standard drug aminophylline. The different concentrations were selected on trial and error basis to tracheal relaxation while a negative control using the vehicle (25 µL) was also employed. The trachea-relaxant effects was assessed against contraction induced by histamine or acetylcholine. The experiments involved exposing the contracted tracheal smooth muscle to different concentration of the extract, aminophylline, or vehicle. The effects of these substances on the contracted trachea were then measured. A decrease in tone was considered indicative of a relaxant effect and was expressed as a percentage change relative to the maximum contraction (Águila Let al 2015).

Results and discussion:

Whole plant of BP was successively extracted with n-hexane, chloroform, acetone and ethanol using soxhelet extractor. The method of extraction was selected on the basis of trial and error basis and most importantly ability to yield of extract. While selection of solvent was determined on the basis of extractive values. N-hexane was used to defat and remove plant pigments, chloroform was selected to get non polar phytochemicals and aglycon moieties present, and acetone was selected to extract semi-polar phytochemicals, whereas ethanol was implement to obtain remaining polar phytochemicals. Examination of these extracts showed presence of prominent phytochemical such as alkaloids, poly phenols, steroids and terpenoids. With growing concern for scientific utilization of plant materials in different forms such extracts, fraction or isolated compounds, its mandatory to standardize these products. Standardization of extracts revealed highest TFC and TPC in ethanol extract exhibiting 19.12 RE mg/g & 24.11 GAE mg/g as compared to other extracts. TFC and TPC contents are usually correlated with basis of biological potentials such as anti—inflammatory and antioxidant. When successively obtained extracts subjected to DPPH and ABTS assay BP-ET extract exhibited best radical scavenging effects with IC_{50} values of 36.39 &25.72 respectively. Furthermore % histamine release in terms of mast cell stabilization exhibited by ethanol extract was10.96 at 50 mg/ml. Finally, among all extracts screened for bronchorelaxent effect against histamine induced contraction, BP-ET showed 94.12% relaxation at 0.6ml.

Conclusion:

Overall finding of present work concludes that, successively obtained whole plant BP extract revealed presence of prominent phytochemicals such as alkaloids, poly phenols, steroids and terpenoids. These phytochemicals attributes for its overall anti-oxidant, mast cell stabilization and broncho-relaxant effects.

Conflict of Interest: The authors declare there is no conflict of interest.

Table 01: Summarized table for phytochemical profile, TPC, TFC, Anti-oxidant and Mast cell stabilization effect of different *Barleria Prionitis* L extracts

Extracts	**BP-HE**	**BP-CH**	**BP-AC**	**BP-ET**
Test for phytoconstituents class				
Alkaloids	-	-	+	+
Amino acid	-	-	-	+
Carbohydrate	-	+	+	+
Glycosides	-	+	+	+
Flavonoids	-	+	+	+
Tannins	-	+	+	+
Steroid	+	+	-	+
Terpenoids	+	+	-	+
Standardization of extracts				
TFC	ND	11.29±1.16	12.15±0.94	19.12±1.08
TPC	ND	09.13±1.10	15.18±0.81	24.11±1.26
***Antioxidant activity IC $_{50}$ values**				
	Ascorbic Acid*	**BP-CH**	**BP-AC**	**BP-ET**
DPPH	14.16 ± 0.84	45.17±1.10	39.86±1.30	36.39 ± 1.29
ABTS	2.101 ± 1.18	48.29±1.03	32.02±0.94	25.72 ± 1.26
% Histamine release measurement for mast cell stabilisation activity *in vitro*				
	DSCG*	**BP-CH**	**BP-AC**	**BP-ET**
50 mg/ml/ 50 µg/ml*	28.6±1.17	35.27±2.23	26.18±1.87	21.59±1.08
50 mg/ml/ 100 µg/ml*	12.08 ±1.80	20.97±2.78	19.08±2.01	10.96±1.91

All the values are mean ± SEM

Table 02: Broncho relaxation studies for BP extracts

% Broncho relaxation against Histamine induced tracheal contraction *in vitro*				
	Aminophylline	**BP-CH**	**BP-AC**	**BP-ET**
0.1	24.02±1.29	10.12±0.98	12±1.10	20.12±1.16
0.2	44.31±1.34	21.10±1.05	18±1.52	31.42±1.20
0.4	72.06±1.40	30.31±1.11	29±1..08	74.12±1.24
0.6	100	37.21±2.12	41±1.55	94.12±1.18
0.8	--	45.98±2.02	56.02±2.86	--

All the values are mean ± SEM

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